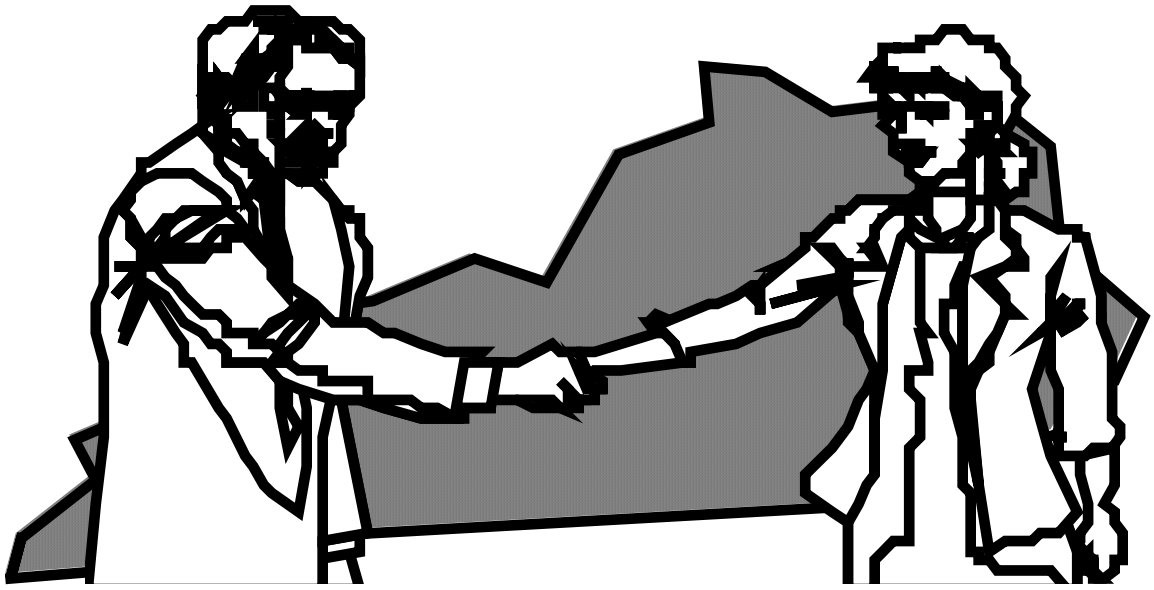
LOCAL PUBLIC HEALTH LABORATORIES OF KENTUCKY



Standard Operating Procedures Manual

Laboratory Procedure Manual

for

Local Public Health Laboratories of Kentucky

CLIA # 18D0686182

All changes or corrections to this manual must be approved by the laboratory director or a technical consultant listed on the multiple sites certificate.

In addition to this manual all current package inserts and operator's manuals should be accessible to the laboratory staff. Package inserts and small manuals may be stored in the pouch provided in Appendix E. Manuals too large for the pouch may be placed in a designated area accessible to all laboratory staff.

Only the laboratory tests addressed in this manual may be performed in your laboratory. Written approval from your laboratory director or technical consultant is necessary prior to startup of any new tests. Generally the Change Form is used as the tool of written approval, see the copy inserted behind this page.

Procedures that are changed, taken out of use, or replaced by another procedure should be dated with the final date used and filed as "Out-of-Use Laboratory Procedures". Store out-of-use laboratory procedures for at least two years in a retrievable location. The CLIA inspector may request their retrieval.

If a particular test system becomes inoperable for any reason, all testing shall cease until the problem is corrected. Quality control shall be performed and documented prior to resuming patient testing. If a backup test system is not available the co-director with the assistance of the laboratory director or technical consultant will decide when and where to refer tests or patients.

The Department for Public Health, Public Health Practice Reference will be adhered to for Medical Management of the patient. Therefore, no normal ranges or panic value criteria will be listed in this procedure manual.

Call 502/ 564-4446 to reach a technical consultant.

Cholesterol
by Accu-Check InstantPlus

Principle

Cholesterol is an important sterol used in metabolism as a precursor of various steriod hormones. Cholesterol monitoring is used in the diagnosis and treatment of disorders involving excessive Cholesterol in blood or lipid and lipoprotein metabolism disorders. The National Institute of Health (NIH) has determined that increased levels of blood cholesterol are a major cause of coronary disease. Determination of total cholesterol in fresh capillary whole blood by means of reflectance photemetry. The lipoprotein complexes will be dissolved by the detergent DONS so that the cholesterol esterase can split the cholesterol ester into cholesterol and the corresponding fatty acid. Cholesterol will be oxidized to 4 cholesten –3 on +H202. Using a reduced indicator, the hydrogen peroxide later reacts to a blue color by means of peroxidase.

Specimen Collection

Obtain a hanging drop of blood by finger puncture and apply to center of the center of the yellow test pad strip. See the appendix "Specimen Collection" for detailed collection techniques.

Equipment and Supplies

Accu-Check InstantPlus Monitor
Accu-Check InstantPlus Cholesterol Test Strips
Accu-Check InstantPlus Cholesterol Control Solution
Skin-Puncture Blood Collection Supplies
Capillary Pipette

Content Use and Storage

1. Perform and document two levels of quality control:

- Before using your monitor for the first time;
- When you open a new box of test strips;
- If you have the cap off the vial of test strips, or if the vial is not tightly capped;
- If you drop the monitor;
- Whenever you want to check the way you are performing your total cholesterol test; and
- When you want to check the performance of the monitor and test strips.

2. Store quality control material according to manufactures instructions. See the product insert for instructions.

3. Do not use past the expiration date.

4. Follow the testing procedure as written below.

5. Two levels of quality control must be within acceptable range prior to reporting any patient test results.

Disposal of Hazards

1. Place all sharps in an appropriate biohazard sharps container and follow acceptable health department disposal practices.

2. Treat all blood, body fluids and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Coding the Accu-Check InstantPlus

1. The Accu-Check InstantPlus monitor must be coded:
   - Whenever a new box of Accu-Check InstantPlus cholesterol test strips is used; and
   - When there is no code number stored in the monitor, i.e., the display shows ---.

Coding the Accu-Check InstantPlus (continued)

2. Turn the monitor ON by pressingthe right hand side of the ON/OFF button.
   - The word CODE should be flashing and the display will show - - - if the monitor has not been coded previously. If the monitor has been coded already, the word CODE will flash and the code number will be displayed on the screen.

3. Take the code strip out of the waxed paper. DO NOT KEEP THE CODE STRIP IN THE TEST STRIP VIAL, because the paint on the code strip may impair the quality of the test strips and lead to incorrect measurements. Always store the code strip at room temperature in the monitor carrying case or with your testing supplies.

4. Coding is done in a two-stop continuous motion. When inserting the code strip, the black marking line on the code strip closest to your thumb should disappear almost completely.
   - With the protection cover closed, steadily insert the code strip in a smooth and continuous motion as far as it will go.
   - Withdraw the code strip immediately. The code is read during insertion and withdrawal of the strip.

Coding is successful if you hear a beep (providing the beeper has been turned on) and the display shows the three-digit code number that is on the vial of test strips you are using.

The code number on the display must match the code number in the gray box that says Accu-Check InstantPlus on the side of the test strips. If the code number is not displayed, repeat steps 1-3.

Test Procedure

1. Make sure the code number on the monitor display is the same as the code number on the gray box on the test strip vial you are using.

2. Remove a test strip from the vial being careful not to touch the yellow test pad or read areas on the back of the strip when hendling a strip.

3. With the monitor on and the protection cover closed, insert the test strip into the slot at the bottom edge of the monitor in one smooth movement until it locks.

Test Procedure (continued)

4. Two beeps are heard and the word CODE stops flashing in the display indicating the test strip code has been read.

5. Collect the patients specimen in the capillary tube with out air bubbles.

6. Open the protective cover and apply a drop of blood from the capillary tube to the yellow test pad.

7. Close the protective cover immediately (within 5 seconds) for the 180 second count down.

8. Record the total cholesterol result. No calculations are needed.

9. To end the test, open the protective cover to remove the test strip. Then close the protective cover.

10. Any uneven coloring in the circular window on the back of the strip is related to the composition of the blood sample and does not interfere with the monitor result.

Reporting Results

Test results are recorded on the patients medical chart using the CH-12 form.

Management Guidelines

Follow specific Department for Health Services Clinical Standard/Guidelines for Medical Management.

Limitations of the Procedure

1. A hanging drop of blood or control solution should be allowed to fall freely onto the test pad of the strip. You will need to retest if more than one drop is applied or if the drop has not completely covered the yellow test pad area.

2. If values obtained are less than 150 mg/dl or greater than 300 mg/dl repeat the test with a new strip and fresh sample.

Limitations of the Procedure (continued)

3. The manufacturer states Accu-Check InstantPlus cholesterol range is 150 – 300 mg/dl.

Instrument Maintenance

The monitor should be cleaned regularly every time a new box of test strips is used. See the user's manual for instructions.

Batteries must be replaced when LOW battery appears on the screen except during the display check when monitor is first turned on.

Problem Solving

Consult the "Troubleshooting Guide" of the User's Manual.

References

Accu-Check InstantPlus User's Manual, Boehringer Manheim Corporation, Indianapolis, IN, 1996.

Accu-Check InstantPlus Cholesterol Test Strips Package Insert, Boehringer Manheim Corporation, Indianapolis, IN, 1996.

Accu-Check InstantPlus Control Solution Package Insert, Boehringer Manheim Corporation, Indianapolis, IN, 1996.

Cholesterol

by Cholestech LDX

Principle

Cholesterol is an important sterol used in metabolism as a precursor of various steroid hormones. Cholesterol monitoring is used in the diagnosis and treatment of disorders involving excessive cholesterol in blood or lipid and lipoprotein metabolism disorders. The National Institute of Health (NIH) has determined that increased levels of blood cholesterol are a major cause of coronary disease. The Cholestech LDX uses a modification of the Allain method. Plasma is separated from the pre-measured heparinized whole blood by a patented filtration system built into the testing cassette.

The plasma is transferred to the reagent pads where cholesterol esterase breaks down the cholesterol esters in the plasma to free cholesterol and the corresponding fatty acids. Cholesterol oxidase in the presence of oxygen, oxidizes free cholesterol to cholestenone and hydrogen peroxide. The hydrogen peroxide oxidizes the color indicator yielding a color endpoint. The intensity of the color produced is proportional to the cholesterol concentration in the specimen. The end color is read by reflectance photometry using the Cholestech LDX.

Equipment and Supplies

Cholestech LDX Analyzer
Cholestech LDX Cholesterol Test Cassettes
Cholestech LDX Capillary Pipettor
Cholestech LDX Capillary Pipette Tubes
Cholestech LDX Controls (Level 1 & 2)
Skin-puncture blood collection supplies

Specimen Collection & Preparation

Obtain a drop of whole blood by finger puncture and fill the Cholestech LDX Capillary Pipette Tube. See Appendix B, “Specimen Collection,” for detailed collection techniques.

Hemolyzed specimens are not satisfactory for testing.

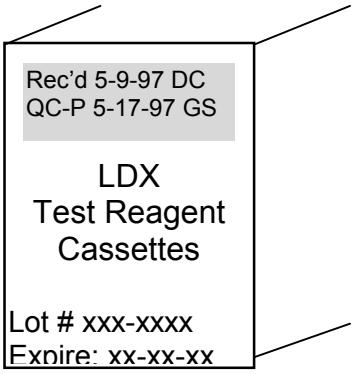
Reagent Preparation and Storage

Cholestech LDX Cholesterol Test Cassettes are stable until the expiration date on the pouch label, when stored in original container at 2-8°C. Allow refrigerated test pouches to reach room temperature prior to testing. Do not open the pouch until immediately before collecting the sample. Protect test cassettes magnetic strip from magnetic fields, do not touch, or allow to get wet.

Keeping Track of Reagent Shipments:

1. When a shipment of reagent cassettes are received into the laboratory mark date received and initials on each box.
2. Prior to patient testing perform and document two levels of quality control on each lot number and each shipment of reagent cassettes received. Mark each box with the following to indicate quality control has been performed and found acceptable: "QC-P", date, and initials below the date received.
3. If the cassette box you are about to open is not marked "QC-P", date, and initials you must perform and document two levels of quality control prior to performing any patient testing.

Example:



Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Cleaning the Cholestech

1. Clean the outside of the Cholestech LDX as needed. See the operator's manual for instruction.

2. Clean the cassette holder tray as needed. See the operator's manual for instruction.

Cholestech System Check

1. Cholestech analyzer automatically runs a self test upon powering up. When self test is completed the display should show "Selftest OK". Consult the operator's manual if another message appears.

2. Allow the Cholestech analyzer to warm up (approximately 5 minutes).

3. After verifying the "Selftest OK", press the RUN button. The drawer will open. The display will read "Load cassette and press RUN".

4. Place the Optics Check Cassette into the cassette holder tray and press RUN.

5. The display will show "Optics Check ##-##-##-##". Record the values and verify they are in acceptable range as printed on the Optics Check Cassette label.

Control Storage and Use

1. Perform and document two levels of quality control

- on each new shipment of cassettes,
- on each lot number of cassettes received,
- if you think cassettes may have been improperly stored,
- anytime the instrument has been moved to a new testing location, and
- anytime the test's performance is in question.

Note: See also page 3 "Keeping Track of Reagent Shipments"

2. The Cholestech controls are stable unopened and refrigerated (2-8°C) until the expiration date listed on the box. Opened vials are stable 30 days refrigerated or at room temperature (36 - 86°C). Do not freeze. Do not use past the expiration date.

3. Allow refrigerated controls to come to room temperature (approximately 10 minutes) prior to testing.

4. Gently mix controls by inverting (at least 8 times). Use the mini-pet pipette to dispense the control material onto the test cassette in step 4 below.

5. Follow the testing procedure as written below.

6. Two levels of quality control must be within acceptable limits prior to reporting any patient test results. Consult the operator's manual for instruction when controls fail.

Test Procedure

1. Verify the daily check and quality control, as described above, have been performed and documented.

2. Remove one cholesterol cassette from the foil pouch, and place it on the working surface with the print side up. Hold cassette by the short sides only. Do not touch the black reaction bar or the magnetic strip.

3. Collect the patient's specimen in the capillary tube without any air bubbles. Specimen must be used within 4 minutes of collection.

4. Dispense the sample into the sample well in the center of the cassette and load into the analyzer without delay.

5. To open the drawer press the RUN button. The instrument will perform the "Selftest" and the drawer will open.

6. Holding the cassette horizontally and not touching the magnetic strip, place the cassette into the analyzer. The magnetic strip should be on the right side.

7. Press RUN, the drawer will close and testing will begin. The display will show "Cholesterol Test Running".

8. The analyzer will beep to signal testing is complete and the test result will appear on the display.

9. Record the test results from the display. No calculations are required.

10. Remove the test cassette.

11. After a test has been performed. Press the RUN button twice to perform a new test.

Procedure Note

The cassette must be loaded into the analyzer for testing without delay once the specimen has been added.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

Limitations of the Procedure

1. Capillary samples must be obtained from a free-flowing puncture site. Excessive squeezing or milking may produce erroneous results.

2. Fluoride, oxalate, or citrate anticoagulants will interfere with the

cholesterol test and should not be used.

3. The manufacturer states Cholestech can read Cholesterol from 100-500 mg/dL. Results outside the stated range will result <100 or >500 mg/dL and the patient should be re-tested by a reference laboratory with a wider testing capability.

Problem Solving

See the operator's manual "Troubleshooting" section.

References

Cholestech LDX Test Cassettes Package Insert, Cholestech Corp. Hayward, CA No date given.

Cholestech LDX Operator's Manual, Cholestech Corp. Hayward, CA No date given.

Cholestech LDX "Quality Control for Laboratory Testing", Technical Bulletin, No. 109, June 1996, Cholestech Corp. Hayward, CA.

Letter from the Cholestech Technical Services Manager (Sue Willson, MT), "Cholestech defines a run as a cassette lot", May 8, 1997.

Fecal Occult Blood

Hemoccult Slide or Hemoccult II Slide

Principle

The Hemoccult test is based on the oxidation of guaiac by hydrogen peroxide to a blue-colored compound. The heme portion of hemoglobin, if present, contains peroxidase activity which catalyzes the oxidation of alpha guaiaconic acid (found in the guaiac paper) by hydrogen peroxide (component of the developer) to form a highly conjugated blue quinone compound.

Specimen Collection

1. A small portion of stool specimen is applied to the test slide using the applicator stick provided by the manufacturer to Box A. Then using the same applicator stick another small portion from a different area of the specimen is added to Box B.
2. Close the flap. Dispose of the applicator stick.
3. Set a timer and wait 3-5 minutes for specimen penetration before developing. Slides may be stored for up to 14 days before developing.

Equipment

Hemoccult Slides
Hemoccult Developer
Applicator Sticks
Timer for 1-4 minutes intervals
Gloves

Storage Instructions

1. Hemoccult Slides should be stored at room temperature. Do not refrigerate or freeze. Protect from heat and light. Do not store with volatile chemicals (e.g., iodine, chlorine, bromine, or ammonia)
2. Do not lift the flap until just before use.
3. Protect the developer from heat and evaporation. Keep the cap tightly closed. The Developer contains and irritant, **avoid skin contact**.

Controls

1. External quality control is not required .
2. Each slide contains internal controls which the manufacturer refers to as the "Performance Monitor". The performance monitor includes both a positive and negative control to be performed and documented with each patient tested.
3. Should the performance monitor fail to react as expected the patient test is invalid and the manufacturer technical services (800-877-6242) should be called for further guidance.

4. A log must be maintained which includes the date of test, analyst initials, patient name or unique identifier, and the results of the performance monitor. A log sheet has been provided, see appendix D.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.
2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department policies.

Testing Procedure/ Development

1. After the test card has set for 3-5 minutes to allow the sample to penetrate, open the back flap and apply two drops of developer to the specimen spots.
2. Read results within 1 minute.
3. When the patient test has been read, perform the performance monitor by adding one drop of developer between the positive and negative areas and read within 10 seconds. Document your results.

Expected Results

1. Any trace of blue color on or at the edge of the specimen (or control) is a positive (+) test result.
2. No blue color equals a negative (-) test result.
3. Control or performance monitors must be performed and documented prior to reporting the patient test results.
4. The controls are performed after the patient test to eliminate possible interference of the blue color of the control when reading the patient test.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

The following patient instructions are recommended by the manufacturer.

- Collect samples three days after the menstrual period, after bleeding hemorrhoids, or blood in the urine.
- Avoid Aspirin, non-steroidal anti-inflammatory drugs for 7 days prior as well as the days of testing..
- Avoid Excessive vitamin C (≥ 250mg/day) for 3 days prior as well as the days of testing..
- Avoid red meat (beef, lamb, liver, processed meats) and raw fruits and vegetables (particularly melons, radishes, turnips, and horseradish) for 3 days prior as well as the days of testing.
- Remove drop-in toilet bowl cleaners form the tank and flush twice before proceeding.
- Collect samples from three consecutive bowel movements or three bowel movements closely spaced in time.
- Protect slides from heat, light, and volatile chemicals (e.g., iodine or bleach).
- Keep cover flap of slides closed when not in use.

Interfering Substances

1. The following have been found to cause false-positive test results:

- Red meat
- Some raw fruits and vegetables
- Preparation of the anal area with iodine containing cleaners
- Ingestion of substances which can irritate the gastrointestinal tract and cause bleeding, such as aspirin, non- steroidal anti-inflammatory drugs, corticosteriods, indomethacin, phenylbutazone, reserpine, anticoagulants, antimetabolites, cancer chemotherapeutic drugs, alcohol in excess, etc.

2. Vitamin C (Ascorbic acid in excess of 250 mg/day) has been found to cause false-negative test results.

Limitations of the Procedure

1. Bowel lesions, including some polyps and colorectal cancers, may not bleed at all or may bleed intermittently. Also, blood, if present, may not be distributed uniformly in the stool specimen. All of which could lead to a false negative test result.
2. This test is a preliminary screen and not intended to replace other diagnostic procedures such as sigmoidoscopy, colonoscopy, barium enema, or other x-ray studies.
3. See interfering substances.
4. See Procedure notes.
5. This test in not intended for specimens other than stool, and may not yield valid results on such specimens.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department (800-877-6242).

Reference

Hemoccult Slide Kit package insert, 12/94.

Fecal Occult Blood
by Seracult Plus Slide

Principle

The Seracult test is based on the oxidation of guaiac by hydrogen peroxide to yield a blue pigment. The hemoglobin component of the whole blood is capable of exerting peroxidase-like activity and is thus able to catalyze the oxidation of alpha-guaiaconic acid. This oxidation produces the visible result of blue coloration.

Specimen Collection

1. **A small portion of stool specimen is applied to the test slide using one end of applicator stick provided. Apply a very thin smear of stool sample to the left window. Obtain another stool sample from a different part of stool wit the same applicator stick. Apply a very thin smear of stool to the right window.**

2. Close cover by inserting flap into tab. Dispose of applicator stick.

3. Slides may be developed immediately after specimen application or may be stored and developed up to 8 days after specimen application.

Equipment and Supplies

Seracult Plus Slides
Seracult Plus Developer
Applicator Sticks
Gloves

Storage Instructions

1. Seracult Plus Slides should be stored between 59°-86°F and are stable until the expiration date printed on each box. Do not refrigerate. Slides should be protected from heat, sunlight, fluorescent light and ultra-violet radiation.

<u>Storage Instructions (continued)</u>

2. Store the Seracult Plus developer between 59°-86°F. Do not refrigerate or freeze. Keep away from heat and light. Developer is stable until the expiration date.

Controls

1. *External quality control is not required.*

2. Each slide contains internal controls which the manufacturer refers to as the "Performance Control Area." The performance control area turns a blue color within 30 seconds to confirm correct performance of reagents.

3. Should the performance control fail to react, the patient test is invalid.

4. A log must be maintained which includes the date of test, analyst initials, patient name or unique identifier, and the results of the performance monitor.

Disposal of Hazards

3. Place all sharps in an appropriate biohazard sharps container and follow applicable health department disposal practices.

4. Treat all blood, body fluids and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Test Procedure/Development

11. To develop specimen test area, lift flap, place two (2) drops of Seracult Plus developer over each smear.

12. Read results in 30-60 seconds.

13. Apply one drop of developer onto Performance Control Area. Blue color should appear within 30 seconds to confirm correct performance of reagents.

Expected Results

1. Any trace of blue color on or at the edge of the specimen is a positive (+) test result.

2. No blue color equals a negative (-) test result.

3. The performance control test must be performed and documented prior to reporting the patient test results.

4. The performance control test should only be performed after the patient specimen tests have been developed and interpreted.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

The following patient instructions are recommended by the manufacturer.

- Collect samples 48 hours following the cessation of hemorrhoidal bleeding or bleeding from the nose, gums, etc., or a menstrual period.
- Stop rectal suppositories or medications before specimen collection.
- Avoid Aspirin, Indomethacin, Phenylbutazone, Cortiosteroids and Reserpine with the consent of a physician for 7 days prior to testing.
- Avoid excessive Vitamin C ($\geq$ 250 mg/dy) for 2 days prior to and during the testing period.
- Avoid ingestion of therapeutic iron 2 days prior to and during the testing period.
- Avoid rare and lightly cooked meats (particularly beef) and raw fruits and vegetables (particularly melons, radishes, turnips, and horseradish) for 3 days prior to and during testing.
- Avoid excessive amounts of alcoholic drinks.
- Collect samples from three consecutive bowel movements and from two different areas of each bowel movement.
- Protect slides from heat, light and volatile chemicals.
- Keep cover flap of slides closed when not in use.

Interfering Substances

1. **The following have been found to cause false positive test results:**
   - Red meat;
   - Some raw fruits and vegetables;
   - Iron rich supplements;
   - Ingestion of substances which can irritate the gastrointestinal tract and cause bleeding, such as aspirin, non-steroidal anti-inflammatory drugs, corticosteroids, indomethacin, phenylbutazone, reserpine, anticoagulants, alcohol in excess, etc.

2. Vitamin C (Ascorbic acid in excess of 250 mg/dy) has been found to cause false negative test results.

Limitations of the Procedure

1. Bowel lesions, including some polyps and colorectal cancers, may not bleed at all or may bleed intermittently. Also, blood, if present, may not be distributed uniformly in the stool specimen. All of which could lead to a false negative test result.

2. This test is a preliminary screen and not intended to replace other diagnostic procedures such as sigmoidoscopy, colonoscopy, barium enema, or other x-ray studies.

3. See interfering substances.

4. See procedure notes.

5. This test is not intended for specimens other than stool, and may not yield valid results on such specimens.

Management Guidelines

Follow specific Department for Health Services Clinic Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department (718) 392-6650 or (718) 392-9271.

References

Seracult Plus Slide Kit package insert, 12/97.

Fecal Occult Blood

ColoCheck Slide

Principle

The ColoCheck test is composed of guaiac impregnated paper which permits sample application to one side, and developement and interpretation on the reverse side. The ColoCheck test is based on the oxidation of phenolic compounds present in the guaiac to quinones resulting in production of the blue color.

Specimen Collection

1. Prior to defecation, the toilet should be flushed. Using one end of the applicator stick collect a small stool sample from the toilet bowl by stabbing the stool.

2. With applicator, apply very thin smear of stool inside box A. Using the same applicator repeat from a different portion of the stool for box B. Discard applicator in the trash after use.

3. Slides may be developed immediately after specimen application or may be stored protected from heat and light and developed up to 12 days after specimen application.

Equipment

ColoCheck Slide
Applicator Sticks
Timer capable of timing both second and minute intervals
Gloves

Storage Instructions

1. ColoCheck Slide should be stored at room temperature. Do not refrigerate or freeze. Protect from heat and light. Do not store with volatile chemicals (e.g., iodine, chlorine, bromine, or ammonia)
2. Protect the developer from heat and evaporation. Keep the cap tightly closed. The Developer contains an irritant, **avoid skin contact**.

Controls

1. External quality control is not required .
2. Each slide contains internal controls which the manufacturer refers to as the "Colocheck Monitor". The Colocheck Monitor includes both a positive and negative control to be performed and documented with each patient tested.
3. Should the Colocheck Monitor fail to react as expected, the patient test is invalid.
4. A log must be maintained which includes the date of test, analyst initials, patient name or unique identifier, and the results of the performance monitor. A log sheet has been provided.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.
2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department policies.

Testing Procedure/ Development

1. Open the perforated window on back side and apply two drops of developer to the thin stool smears boxes.
2. Read results after 30 seconds and within 2 minutes.
3. When the patient test has been read, perform the Colocheck Monitors by adding two drop(s) of developer between the positive and negative boxes and read within 30 seconds to 2 minutes. Document your results.

Expected Results

1. Any trace of blue color within the specimen (or control) application area is a positive (+) test result.
2. No blue color equals a negative (-) test result.
3. ColoCheck Monitors must be performed and documented prior to reporting the patient test results.
4. The ColoCheck Monitors are performed after the patient test to eliminate possible interference of the blue color of the control when reading the patient test.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

The following patient instructions are recommended by the manufacturer.

- Collect samples three days after the menstrual period, after bleeding hemorrhoids, or blood in the urine.
- Avoid Aspirin, non-steroidal anti-inflammatory drugs for 7 days prior as well as the days of testing..
- Avoid Excessive vitamin C (≥ 250mg/day) for 2 days prior as well as the days of testing..
- Avoid red meat (beef, lamb, liver, processed meats) and raw fruits and vegetables (particularly cantaloupe, prunes, red radishes, turnips, and horseradish, brocolli, parsnips, cauliflower) for 2 days prior as well as the days of testing.
- Remove drop-in toilet bowl cleaners from the tank and flush twice before proceeding.
- Collect samples from three consecutive bowel movements or three bowel movements closely spaced in time.
- Protect slides from heat, light, and volatile chemicals (e.g., iodine or bleach).

Interfering Substances

1. The following have been found to cause false-positive test results:

- Red meat
- Some raw fruits and vegetables
- Preparation of the anal area with iodine containing cleaners
- Ingestion of substances which can irritate the gastrointestinal tract and cause bleeding, such as aspirin, non- steroidal anti-inflammatory drugs, corticosteriods, indomethacin, phenylbutazone, reserpine, anticoagulants, antimetabolites, cancer chemotherapeutic drugs, alcohol in excess, etc.

2. Vitamin C (Ascorbic acid in excess of 250 mg/day) has been found to cause false-negative test results.

Limitations of the Procedure

1. Bowel lesions, including some polyps and colorectal cancers, may not bleed at all or may bleed intermittently. Also, blood, if present, may not be distributed uniformly in the stool specimen. All of which could lead to a false negative test result.
2. This test is a preliminary screen and not intended to replace other diagnostic procedures such as sigmoidoscopy, colonoscopy, barium enema, or other x-ray studies.
3. See Interfering Substances.
4. See Procedure notes.
5. This test in not intended for specimens other than stool, and may not yield valid results on such specimens.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert.

Reference

ColoCheck Slide package insert, 9/91.

Fecal Occult Blood

ColoCheck Tape

Principle

The ColoCheck test is composed of guaiac impregnated paper which permits sample application to one side, and developement and interpretation on the reverse side. The ColoCheck test is based on the oxidation of phenolic compounds present in the guaiac to quinones resulting in production of the blue color.

Specimen Collection

1. Tear a segment of paper from the dispenser. Note that Positive and Negative Monitors are printed on one side of the paper segment.
2. A small portion of stool specimen (very thin smear) is applied to the tape on the side opposite the Monitors. Do not smear the specimen over the area where the Monitors are printed.

Equipment

ColoCheck tape
Applicator Sticks
Timer capable of timing both second and minute intervals
Gloves

Storage Instructions

3. ColoCheck tape should be stored at room temperature. Do not refrigerate or freeze. Protect from heat and light. Do not store with volatile chemicals (e.g., iodine, chlorine, bromine, or ammonia)
4. Protect the developer from heat and evaporation. Keep the cap tightly closed. The Developer contains an irritant, **avoid skin contact**.

Controls

5. External quality control is not required .
6. Each slide contains internal controls which the manufacturer refers to as the "Colocheck Monitor". The Colocheck Monitor includes both a positive and negative control to be performed and documented with each patient tested.
7. Should the Colocheck Monitor fail to react as expected, the patient test is invalid.
8. A log must be maintained which includes the date of test, analyst initials, patient name or unique identifier, and the results of the performance monitor. A log sheet has been provided.

Disposal of Hazards

3. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.
4. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department policies.

Testing Procedure/ Development

4. Turn the tape segment over and apply two drops of developer to the thin stool smear.
5. Allow the smear to air dry.
6. Read results after 30 seconds and within 2 minutes.
7. When the patient test has been read, perform the Colocheck Monitors by adding two drop(s) of developer between the positive and negative boxes and read within 30 seconds to 2 minutes. Document your results.

Expected Results

5. Any trace of blue color within the specimen (or control) application area is a positive (+) test result.
6. No blue color equals a negative (-) test result.
7. ColoCheck Monitors must be performed and documented prior to reporting the patient test results.
8. The ColoCheck Monitors are performed after the patient test to eliminate possible interference of the blue color of the control when reading the patient test.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

The following patient instructions are recommended by the manufacturer.

- Collect samples three days after the menstrual period, after bleeding hemorrhoids, or blood in the urine.
- Avoid Aspirin, non-steroidal anti-inflammatory drugs for 7 days prior as well as the days of testing..
- Avoid Excessive vitamin C (≥ 250mg/day) for 2 days prior as well as the days of testing..
- Avoid red meat (beef, lamb, liver, processed meats) and raw fruits and vegetables (particularly cantaloupe, prunes, red radishes, turnips, and horseradish, brocolli, parsnips, cauliflower) for 2 days prior as well as the days of testing.
- Remove drop-in toilet bowl cleaners form the tank and flush twice before proceeding.
- Collect samples from three consecutive bowel movements or three bowel movements closely spaced in time.
- Protect slides from heat, light, and volatile chemicals (e.g., iodine or bleach).

Interfering Substances

3. The following have been found to cause false-positive test results:

- Red meat
- Some raw fruits and vegetables
- Preparation of the anal area with iodine containing cleaners
- Ingestion of substances which can irritate the gastrointestinal tract and cause bleeding, such as aspirin, non- steroidal anti-inflammatory drugs, corticosteriods,

indomethacin, phenylbutazone, reserpine, anticoagulants, antimetabolites, cancer chemotherapeutic drugs, alcohol in excess, etc.

4. Vitamin C (Ascorbic acid in excess of 250 mg/day) has been found to cause false-negative test results.

Limitations of the Procedure

6. Bowel lesions, including some polyps and colorectal cancers, may not bleed at all or may bleed intermittently. Also, blood, if present, may not be distributed uniformly in the stool specimen. All of which could lead to a false negative test result.
7. This test is a preliminary screen and not intended to replace other diagnostic procedures such as sigmoidoscopy, colonoscopy, barium enema, or other x-ray studies.
8. See Interfering Substances.
9. See Procedure notes.
10. This test in not intended for specimens other than stool, and may not yield valid results on such specimens.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert.

Reference

ColoCheck Tape package insert, 10/91.

Fecal Occult Blood
by HEMA SCREEN

Principle

The HEMA SCREEN test is based on the oxidation of guaiac by hydrogen peroxide to yield a blue pigment. The hemoglobin component of the whole blood is capable of exerting peroxidase-like activity and is thus able to catalyze the oxidation of alpha-guaiaconic acid. This oxidation produces the visible result of blue coloration.

Specimen Collection

1. Prior to defecation, the toilet should be flushed. Using one end of the applicator stick collect a small stool sample from the toilet bowl by stabbing the stool.

2. With applicator, apply very thin smear of stool inside Oval where indicated with Roman numeral I. Using the same applicator repeat from a different portion of the stool for Oval II. Discard applicator in the trash after use.

3. Slides may be developed immediately after specimen application or may be stored protected from heat and light and developed up to 14 days after specimen application.

Equipment and Supplies

HEMA SCREEN Slides
HEMA SCREEN Developing Solution
Applicator Sticks
Gloves

Storage Instructions

1. HEMA SCREEN Slides should be stored between 59°-86°F and are stable up to three years from date of manufacture. Do not refrigerate or freeze. Slides should be protected from heat, sunlight, fluorescent light and ultra-violet radiation.

2. Store the HEMA SCREEN developer between 59°-86°F. Do not refrigerate or freeze. Keep away from heat, humidity, and light. Developer is stable up to three years from date of manufacture.

Controls

1. External quality control is not required.

2. Each slide contains internal controls which the manufacturer refers to as the "Performance Standards." The positive performance standard turns a blue color within 30 seconds to confirm correct performance of reagents. The negative performance standard remains colorless.

3. It is important that the Performance Standards be developed after specimens to avoid interference or prejudice of the test interpretation. Add one drop of developer directly onto control area (between the positive and negative performance standards). Read results within 30 seconds.

4. Should the performance control fail to react, the patient test is invalid.

5. A log must be maintained which includes the date of test, analyst initials, patient name or unique identifier, and the results of the performance monitor.

Disposal of Hazards

1. Place all sharps in an appropriate biohazard sharps container and follow applicable health department disposal practices.

2. Treat all blood, body fluids and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Test Procedure/Development

1. To develop specimen test area, open perforated section marked 1 and 2 on back of slide, place two (2) drops or more drops of HEMA SCREEN developing solution to exposed paper.

2. Read results after 30 seconds and before two minutes.

3. Apply one drop of developer onto Performance Control Area. Blue color should appear within 30 seconds to confirm correct performance of reagents.

Expected Results

1. Any trace of blue color on or at the edge of the specimen is a positive (+) test result.

2. No blue color equals a negative (-) test result.

3. The performance control test must be performed and documented prior to reporting the patient test results.

4. The performance control test should only be performed after the patient specimen tests have been developed and interpreted.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

The following patient instructions are recommended:

- Collect samples 48 hours following the cessation of hemorrhoidal bleeding or bleeding from the nose, gums, etc., or a menstrual period.
- Stop rectal suppositories or medications before specimen collection.

- Avoid Aspirin, Indomethacin, Phenylbutazone, Cortiosteroids and Reserpine with the consent of a physician for 7 days prior to testing.
- Avoid excessive Vitamin C ($\geq$ 250 mg/dy) for 2 days prior to and during the testing period.
- Avoid ingestion of therapeutic iron 2 days prior to and during the testing period.
- Avoid rare and lightly cooked meats (particularly beef) and raw fruits and vegetables (particularly melons, radishes, turnips, and horseradish) for 3 days prior to and during testing.
- Avoid excessive amounts of alcoholic drinks.
- Collect samples from three consecutive bowel movements and from two different areas of each bowel movement.
- Protect slides from heat, light and volatile chemicals.
- Keep cover flap of slides closed when not in use.

Interfering Substances

1. The following have been found to cause false positive test results:
   Red meat;
   Some raw fruits and vegetables;
   Iron rich supplements;
   Ingestion of substances which can irritate the gastrointestinal tract and cause bleeding, such as aspirin, non-steroidal anti-inflammatory drugs, corticosteroids, indomethacin, phenylbutazone, reserpine, anticoagulants, alcohol in excess, etc.

2. Vitamin C (Ascorbic acid in excess of 250 mg/dy) has been found to cause false negative test results.

Limitations of the Procedure

1. Bowel lesions, including some polyps and colorectal cancers, may not bleed at all or may bleed intermittently. Also, blood, if present, may not be distributed uniformly in the stool specimen. All of which could lead to a false negative test result.

2. This test is a preliminary screen and not intended to replace other diagnostic procedures such as sigmoidoscopy, colonoscopy, barium enema, or other x-ray studies.

3. See interfering substances.

4. See procedure notes.

5. This test is not intended for specimens other than stool, and may not yield valid results on such specimens.

Management Guidelines

Follow specific Department for Health Services Clinic Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department 1-800-722-7505.

References

HEMA SCREEN Slide Kit package insert, 3/99.

Glucose, Whole Blood
by Precision QID

Principle

Disorders of carbohydrate metabolism are often detected and monitored by glucose testing. The Precision QID is a battery operated biosensor developed for rapid measurement of D-glucose in fresh capillary blood. It employs a electrochemical detection technique and is based on the glucose oxidase test method.

Each test strip features an electrode containing the enzyme glucose oxidase. When a blood drop is applied to the target area of the dry test strip, the glucose oxidase catalyzes the oxidation of glucose in the drop to produce gluconic acid. During the reaction, electrons are transferred by an electrochemical mediator to the electrode surface. This in turn generates a current that is measured by the Precision QID Sensor. The size of the current generated is proportional to the amount of glucose present in the blood drop, thus giving an accurate reading of the blood glucose concentration.

Specimen Collection

Obtain a drop of fresh capillary whole blood by finger puncture and apply it directly to the pre-loaded Precision test strip. See the appendix "Specimen Collection" for detailed collection techniques.

Equipment and Supplies

Precision QID Sensor (meter)
Precision QID test strips (with Calibrator strip)
Medisense Glucose Control Solutions (levels high & low)
Skin-puncture blood collection supplies

Reagent Preparation and Storage

1. Precision QID test strips are sealed in individual foil packets. Store the test strips at a temperature between 39-86°F (4-30°C) and out of direct sunlight. Do not refrigerate or freeze.

2. Each box of Precision QID test strips are packaged with a box specific calibration strip and package insert. Save both the calibration strip and the package insert until all the test strips have been used.

3. When properly stored, the unopened test strips are stable until the expiration date printed on the foil packet. (Strips expire the last day of the printed month, eg. 12/97 would expire 12/31/97.)

4. Once removed from the foil packet, strips should be promptly used.

5. Do not use test strips that have gotten dirty, wet, bent, scratched or damaged in any way.

6. Do not smear blood onto the test strip target area.

7. Do not reuse test strips.

8. Do not attempt to cut the test strips.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Calibration

The calibration strip packaged in each box of test strips contains the calibration data specific for that box of Precision QID test strips. To obtain an accurate blood glucose reading, the Precision QID Sensor must be calibrated for each new box of test strips. Use only the calibrator that is packaged with the test strips currently in use.

1. Check that the three-character calibration code and the lot number printed on the calibrator match those printed on the reagent strips package insert (see package insert front side, top of second column). Check that the lot

number printed on the test strips foil packets match the one printed on the calibration strip.

2. Insert the contact bars of the calibrator into the sensor (meter). Make sure the writing is facing up.

3. The sensor (meter) automatically comes on and displays "**88.8**", alternating with "**CAL**", and the three-character calibration code.

4. Check that the calibration code matches the calibration code printed on the calibrator.

5. Press and release the blue button to turn the sensor (meter) off. Calibration is now complete.

6. Remove the calibrator and store in a safe place. Do not discard the calibrator strip until the test strips are all used up.

Control Storage and Use

1. Perform and document two levels of quality control (levels high and low)
- whenever a new box of test strips is opened,
- whenever the test strips may have been improperly stored, and
- anytime the patient test results are in question.

1. In the instances described in number 1, two levels of quality control must be within acceptable limits prior to reporting any patient test results. The expected values are found on the test strip package insert currently in use. (See the package insert front side, second column, after the calibration data.)

2. Medisense glucose control solutions are stored at 39-86°F (4-30°C). Do not refrigerate or freeze. Control solutions work best at 64 -86°F and 20-80% humidity.

3. Do not use past the printed expiration date. Control solutions are stable for 30 days from the date opened or the printed expiration date, which ever occurs first. Write the date of opening on the control vial and discard after 30 days. Make sure the lid is returned to the control vial and fully

tightened after each use.

4. Invert the control vials three to four times to ensure thorough mixing before use.

5. Avoid touching the control vial tip to the test strip.

6. Follow the testing procedure as written below.

Test Procedure

See the User's Manual for an illustrated procedure.

1. Perform calibration and quality control as indicated above.

2. Gather the supplies required for specimen collection.

3. Tear open the foil packet at the slit to expose the contact bars. Without touching the contact bars or exposing the target test area, insert the test strip into the sensor (meter).

4. Gently push the test strip in until it stops, the display will show "**88.8**" alternating with "**CAL**", the calibration code, and then "**rdy**".

5. Check the calibration code and make sure it matches the code printed on the test strip foil packet. If these number do not match the sensor is improperly calibrated and must be calibrated and controlled prior to patient testing.

6. Remove the test strip packet and set it aside for later use in discarding the used test strip.

7. Obtain the capillary whole blood specimen and apply a hanging drop to the test pad target area while the display reads "**rdy**". The blood drop will be drawn into the target area and the test will automatically begin when enough blood is present. When the display shows "**---**", move the patient's finger away form the test strip.

Should the test fail to start, a second drop of blood may be applied to the target area within 30 seconds of the first drop. If the test still fails to

start or more than 30 seconds have elapsed, discard the test strip and repeat the test.

8. Following "**---**", the display will show "**ctd**" , and then begin a 20 second count down to the test results appear. Record the test results on the CH-12 and press the blue button to turn the sensor (meter) off.

9. Discard the test strip by replacing the foil packet and pulling the strip straight out of the meter.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

1. If the test results appear to be inconsistent with the patient's symptoms or are less than 50 mg/dL or greater than 300 mg/dL, the manufacturer recommends recalibrating the sensor (meter) and repeating the test using a new test strip.

2. Error Messages:

- A value of "**low**" means the glucose level is at or below the detection limit of 20 mg/dL.
- A value of "**high**" means the glucose level is at or above the detection limit of 600 mg/dL.
- An error code of ["**rdy**", "**---**", "**--**", "**-**"] means the glucose level is at or above the detection limit of 600 mg/dL.
- An error code of "**Err**" near the end of the count down means the glucose level is at or above the detection limit of 600 mg/dL.
- An error code of "**Err**" during the count down indicates improper insertion of the strip or a damaged strip.

Limitations of Procedure

1. The Precision QID is designed for the use of fresh whole capillary blood. DO NOT USE serum or plasma patient samples.

2. Extremes in humidity (less than 10% or greater than 90%) may cause

false test results.

3. Extremes in hematocrit (less than 39% HCT or greater than 60%) may cause false test results. Not recommended for neonatal blood samples.

4. Acetaminophen at or greater than 10 mg/dL and salicylate at toxic levels may yield false low results.

5. Patients undergoing oxygen therapy may yield falsely lower results.

6. Patients experiencing sever dehydration or severely hypotensive, in shock or in hyper-glycemic-hyperosomolar state may yield false low results.

7. Extremely high levels of the following substances as listed below do not affect test results:

8. Uric acid 20 mg/dL, ascorbic acid 3 mg/dL, cholesterol 500 mg/dL, and triglycerides 3000 mg/dL.

Problem Solving

See operator's manual chapter seven, “Display messages”.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards for Medical Management.

Instrument Maintenance

A “**Lo bAt**” error message indicates the meter’s battery is low, contact Medisense, within 24 hours, for a free replacement meter (800/527-3339).

References

Precision QID User's Manual, Medisense, undated.
Precision QID Test Strips, product insert, Medisense, Waltham , MA 02154, 1995.
Medisense Glucose Control Solution, product insert, Medisense, Waltham , MA 02154, 1995.

Glucose, Whole Blood
by Accu-Chek Advantage

Principle

Disorders of carbohydrate metabolism are often detected and monitored by glucose testing. The Accu-Chek Advantage is a battery operated photometer which employs the electrochemical principle of biamperometry.

The enzyme glucose dehydrogenase converts the glucose in a blood sample to gluconolactone. This reaction liberates an electron that reacts with a coenzyme electron acceptor, the oxidized form of the mediator hexacyanoferrate (III), forming the reduced form of the mediator, hexacyanoferrate (II). The monitor applies a voltage between two identical electrodes, which causes the reduced mediator formed during the incubation period to be reconverted to oxidized mediator. This creates a small current that is read by the monitor.

Specimen Collection

Obtain a drop of whole blood by finger puncture and apply it directly to the Accu-Chek Advantage test strip. See the appendix "Specimen Collection" for detailed collection techniques.

Equipment and Supplies

Accu-Chek Advantage monitor
Accu-Chek Check Strip
Advantage Test Strips (with Advantage Code Key)
Advantage Glucose Control Solutions
Skin-puncture blood collection supplies

Reagent Preparation and Storage

- Keep the test strips in the original capped vial.
- Tightly replace the vial cap immediately after removing a test strip.
- Store the Accu-Chek Check Strip in a dry place, away from light. Do not freeze.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Calibration

The Advantage Code Key contains all the calibration data for one lot number of test strips. The Advantage Code Key chip is packaged in the Advantage Reagent Strip box. Change the Code Key each time a new vial of test strips is opened.

1. Make sure monitor is turned off. Turn monitor over so you are looking at the back. Remove old Code Key if one is installed.

2. Insert new Code Key until it snaps in place.

3. Turn monitor ON. A 3-digit code number will appear. This number must match the code number on your vial of test strips. If it does not, repeat steps 1-3.

Advantage Quality Control Checks

The Quality Control Checks consists of two Glucose Control solutions and an Advantage Quality Control Check Strip. The Quality Control Checks should be used:

- before using the monitor for the first time
- whenever a new vial of test strips is opened
- if the cap has been left off the vial of test strips
- when the monitor has experienced trauma such as being dropped
- anytime the operator suspects a problem

Control Storage and Use

1. Easy glucose control solutions are stored at room temperature. Do not freeze.

2. Do not use past the printed expiration date. Control solutions are stable 3 months from the date opened or the printed expiration date, which ever occurs first.

3. Follow the testing procedure as written below.

Test Procedure

See the Accu-Chek Advantage User's Manual for an illustrated procedure.

1. Turn the monitor on and verify calibration by checking the displayed three digit number against the code on the test strip vial.

2. When the test strip symbol flashes on the display, the monitor is ready to accept a test.

3. Remove a test strip from the vial and replace the lid immediately.

4. Within 30 seconds, gently insert tests strip (yellow target area facing up) into test strip guide. Once strip is properly inserted, a blood drop symbol flashes on the display.

5. Obtain blood sample. Make sure you have a hanging drop of blood before you apply it to test strip.

6. Touch drop of blood to center of yellow target area. Make sure the target area is completely covered and no yellow mesh is visible. Do not smear blood with your finger on the target area.

7. When blood is applied correctly to strip, a box rotates on the display until measurement is completed. When the blood glucose result is displayed, record the result.

8. Remove test strip from the monitor and properly discard. When test strip is removed, the strip symbol flashes indicating the monitor is ready to accept another strip.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

1. Do not use expired test strips or control solutions.

2. The monitor must be in the off position when changing the Code Key.

3. Very small amounts of blood may give you an error message or an inaccurate result. Ensure that enough blood to cover the strip is obtained.

Problem Solving

See Accu-Chek Advantage User's Manual "Troubleshooting" section.

Limitations of Procedure

1. This procedure is limited to the use of fresh capillary blood only.

2. Galactose in excess of 10 mg/dL may give falsely elevated results.

3. Hematocrit levels below 25% and above 55% may cause inaccurate test results with glucose concentrations above 200 mg/dL.

4. This method has been tested with neonate blood. It is recommended that neonate glucose values below 50 mg/dL be verified by a reference laboratory.

5. Elevated uric acid levels may cause falsely elevated results in some patients.

6. Linearity as stated by the manufacturer is 10 - 600 mg/dL.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards for Medical Management.

Instrument Maintenance

1. Clean the outside of the monitor with a soft cloth that has been slightly dampened with 70% alcohol or a solution of 1 part bleach and 10 parts water. Do not get moisture into the Advantage Code Key slot or the test strip guide.

2. Keep monitor free of dust.

3. Protect monitor from extremes in temperature and humidity.

4. Handle with care. If monitor is dropped, perform quality control check.

5. Do not test with monitor placed on warm or cool surfaces.

6. Follow Accu-Chek Advantage User's Manual instructions regarding battery replacement.

References

Accu-Chek Advantage Reagent Strips, product insert, Boehringer Mannheim, Indianapolis, IN, 1996.

Accu-Chek Advantage Glucose Control Solutions, product insert, Boehringer Mannheim, Indianapolis, IN, 1995.

Glucose, Whole Blood
by SureStepPro System

Principle

Disorders of carbohydrate, fat, and protein metabolism are managed and monitored utilizing whole blood glucose testing. The SureStep is a battery operated photometer which utilizes photoelectronics to measure whole blood glucose in conjunction with a dry regent strip. The testing method used is a glucose oxidase/peroxidase/a naphthalene sulfonic acid salt/hydrazone reaction.

When blood is placed on the pink test square of the SureStep test strip, whole blood glucose is broken down by glucose oxidase and oxygen resulting in gluconic acid and hydrogen peroxide. Peroxidase causes the hydrogen peroxide, when combined with oxygen, to react with dyes that produce a (naphthlalene) blue color. The darker the blue color, the higher the glucose level.

Specimen Collection

Obtain a drop of fresh capillary whole blood by finger puncture and apply it directly to the SureStep test strip. See the appendix "Specimen Collection" for detailed collection techniques.

Equipment and Supplies

SureStep blood glucose meter
SureStep Pro test strip container
SureStep Pro control solutions
Specimen collection supplies

Reagent Preparation and Storage

1. SureStep Pro test strips are to be stored in their original container in a cool, dry place below 86°F. Keep away from heat and direct sunlight. Do not refrigerate or freeze.

2. After removing a test strip for the container, immediately replace the cap. Keep container capped to avoid moisture and light exposure. Test strip contact with air or moisture may cause inaccurate results.

3. Date vial when first opened. Reagent strips are stable until four months after opening or printed expiration date, whichever comes first.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids and used testing apparatus as potentially infectious waste and follow health department disposal policies.

Checking the System

The SureStep system does not require a check strip. The system is checked automatically every time it is turned on. The **CODE** number is used to calibrate the system. You must match the code number on the meter display to the code number on the test strip container you are using. If the code numbers do not match you will get an inaccurate reading.

Set the code number before using the meter for the first time and every time you open a new test strip container using the following procedure:

1. Remove the SureStep Pro test strip container from the shipping box.

2. Turn the SureStep blood glucose meter ON by pressing the blue button.

3. Code the SureStep blood glucose meter by pressing the green **C** button until the code number on the SureStep Pro test strip container number matches meter display.

4. Verify the code number on the container matches the code number on the display.

Control Storage and Use

The SureStep Pro High and Low controls are used to verify that the SureStep blood glucose meter and the SureStep Pro test strips are working together properly. You should get results within the expected range printed on the test strip package.

1. Perform and document two levels of quality control:

    - every day of testing,
    - whenever a new vial of test strips is opened,
    - anytime the patient test results are in question,
    - if you drop the meter.

2. Two levels of quality control must be within acceptable limits prior to reporting any patient test results.

3. Do not store above 86°F. Do not refrigerate or freeze.

4. Date vial when first opened. SureStep controls are stable until three months after opening or printed expiration date, whichever comes first.

5. Gently shake the control vials to ensure thorough mixing before use.

6. Avoid touching the control vial tip to the test strip.

7. Follow the testing procedure below, substituting control solution for the drop of blood.

Test Procedure

See the User's Manual for an illustrated procedure.

1. Turn the SureStep blood glucose meter ON by pressing the blue button. The latest test result appears followed by the **CODE** symbol and number. If the code numbers do no match, repeat steps 1-4 in the Checking the System section.

2. Remove a SureStep Pro test strip from the container. Replace the cap immediately.

3. Obtain a blood sample using appropriate skin puncture collection supplies.

4. A flashing hand with a drop of blood from a finger symbol appears over a strip, and a test strip appears. The meter is ready for testing.

5. Apply blood to the **center of the pink square testing area** on the SureStep Pro test strip. The pink square quickly absorbs the blood.

6. Check the confirmation dot on the back of the test strip, which should be completely blue for an accurate test. Turn the strip over so the pink square test area is facing up.

7. Insert the test strip into the meter within two minutes of applying blood. A flashing clock will appear, and results will appear in about thirty seconds.

8. Press the blue button to turn OFF the meter.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 .

Procedure Notes

1. Only use test strips which match the UNUSED color block found on the SureStep Pro test strip container.

2. Avoid testing in extreme temperatures.

3. Use caution when interpreting neonatal blood glucose results which are less than 50mg/dL.

Problem Solving

See User's Manual "Troubleshooting" section.

Limitations of Procedure

1. Use only fresh whole blood. Do not use serum or plasma.
2. Use an adequate amount of blood--just enough to cover the pink test square.
3. Extremes in hematocrit (<39% and > 60% HCT) cause false test results.
4. Venous and capillary blood may differ in glucose concentration by as much as 70 mg/dL depending upon the time of blood collection after food intake. Shock, administration of vasoactive agents, and other factors affecting the peripheral circulation may also cause discrepancies between venous and capillary glucose results.
5. Blood glucose results obtained with the SureStep Pro test strips may be affected if excessive water loss or dehydration occurs.
6. The range of SureStep Brand Meters is 0-500 mg/dL. Above this range the meters read HIGH.
7. Highly lipemic (fatty) blood samples, up to 3000 mg/dL triglycerides, have no significant effect on results.
8. Ascorbic acid, at concentrations of 3 mg/dL, has no significant effect on results.

Instrument Maintenance

See the User's Manual for an illustrated procedure.

1. Clean the test strip holder and lens area whenever the test area looks dirty or any time an Er5 appears on the display. Use the following procedure:

    a Remove the test strip holder by pressing down on the top of the test strip holder an d sliding it away from the meter.
    b Clean the test strip holder by wiping it gently with a cotton swab or cloth dampened with a 10% bleach solution in water. Wipe the gray area on the inside cover. Wash both sides of the base; carefully clean around the hold in the base.

c Rinse thoroughly with water to remove any residual bleach. Dry the test strip holder completely with a soft cloth or lint-free tissue.

d Using a cotton sway or a cloth dampened with a 10% bleach solution, wipe the lens area. Wipe this area even if it does not appear to be dirty. Use a cloth moistened with water to remove any residual bleach. Dry the test strip holder completely with a soft cloth or lint-free tissue. Be careful not to scratch the lens area or get water inside the meter.

e Replace the test strip holder by sliding it into the meter and press down at the strip insertion point until you hear a click. Make sure the test strip holder is firmly in place and does not extend beyond the meter.

f If Er5 message occurs, see the User's Manual for correction.

2. Replace the battery according to the User's Manual instructions.

Management Guidelines

Follow specific Department for Public Health Clinical Services Standards for Medical Management.

References

Lifescan SureStep Owner's Manual, Lifescan, Inc., Johnson and Johnson, Co., 1995, Milipitas, CA.

Lifescan SureStep Reagent Strips (SureStep Pro) product insert, Lifescan, Inc., Johnson and Johnson, Co., 1996, Milipitas, CA.

Lifescan SureStep Pro Control Solution product insert, Lifescan, Inc., Johnson and Johnson, Co., 1996, Milipitas, CA.

Glucose, Whole Blood
by Glucometer Elite

Principle

Disorders of carbohydrate metabolism are often detected and monitored by glucose testing. The Glucometer Elite is a battery operated photometer which is based on an electrode sensor technology.

The Glucometer Elite blood glucose test is based on measurement of electrical potential caused by the reaction of glucose with the reagents on the electrode of the strip. The blood sample is drawn into the tip of the Test Strip through capillary action. Glucose in the sample reacts with glucose oxidase and potassium ferricyanide. Electrons are generated producing a current which is proportional to the glucose in the sample.

Specimen Collection

Obtain a drop of whole blood by finger puncture and apply the test strip to the drop of blood until after the meter "beeps". See appendix "Specimen Collection" for detailed collection techniques.

Equipment and Supplies

Glucometer Elite Meter
Glucometer Elite test strip (with Code Strip)
Glucometer Elite normal Control and Check Strip
Skin puncture blood collection supplies

Reagent Preparation and Storage

1. Glucometer Elite Check Strip is stored in a plastic box**.**

2. Glucometer Elite test strips are foil wrapped. Store away from direct sunlight and away from extreme temperatures. Use test strip immediately after opening foil packet.

3. Protect from excessive heat, humidity or freezing temperatures.

4. Test strips should be allowed to reach room temperature prior to use.

5. Avoid touching either end of the test strip.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Calibration

The Glucometer Elite Code Strip contains all the calibration data for the Glucometer Elite. The Glucometer Elite Code Strip is provided with each package of Test Strips. Each package of Test Strips contains its own Code Strip.

1. Discard the old Code Strip when the Test Strips from previous box are all used.

2. Carefully tear open the Code Strip packet and remove Code Strip. Save the packet to store the Code Strip. **AVOID TOUCHING THE METER END OF THE CODE STRIP.**

3. Insert the Code Strip into the test Slot at the end of the meter until it comes to a full stop. A double "beep" will sound, and the Function Number (e.g., F-4) will appear in the display.

4. The Function Number appearing in the display **MUST** match the number appearing on 1) the Code Strip; 2) the front of the Code Strip packet; and 3) the back of the Test Strip packet.

5. The Meter must be coded again when a new carton of Test Strips is used. Use the new Code Strip found in the new carton of strips following the above procedure.

The Glucometer Elite Check Strip Test

The Check Strip Test should be performed:

a. on all new meters prior to any patient testing
b. after the installation of new batteries
c. anytime the operator wants to confirm the correct operation of the meter

Glucometer Elite Check Strip Test Procedure

1. Remove Check Strip from box. **AVOID TOUCHING THE METER END OF THE CHECK STRIP.**

2. Insert the Check Strip fully into the Meter with the tab toward the tope of the meter. A double "beep" will sound and the power will automatically turn on. The Check Strip result will then appear in the Display Window.

3. If the display reading is within the range listed on the Check Strip label insert, the meter is functioning properly. If not, refer to the Problem Solving chart in Section 5 of the owner's manual.

4. Carefully remove the check Strip from the meter and replace it in the plastic box.

Control Storage and Use

1. Perform and document Normal Control Test prior to the first test of the day and whenever a new carton of Test Strips are opened.

2. Glucometer Elite Normal Control solution is stored at room temperature (59-86° F). If solution is cold, allow to come to room temperature prior to use.

3. Use before the unopened expiration date shown on the bottle and within six (6) months after first opening.

4. Follow the testing procedure as written below.

5. If the Control Test does not fall within the stated range on the Control Range Card in the carton of Test Strips, the meter is not functioning properly. See Section 5 of the User Guide and Control Package insert for help.

Test Procedure

See the User's Manual for an illustrated procedure.

1. Remove foil packets from carton and tear off single packet.

2. Carefully **PEEL TO THE LINE.** Fold back the foil ends to expose the meter end of the test strip. **AVOID TOUCHING EITHER END OF THE TEST STRIP.**

3. Hold the test end of the Test Strip **between the foil,** and, with the tab facing out, insert the strip fully into the meter.

4. A "beep" will sound the function Number (F#) and previous test result will begin flashing on the Display Window. Remove the foil from the strip. (Be sure to have the foil to remove the used Test Strip from the meter.)

5. Touch and hold the Test End (tip) of the Test Strip to the hanging drop of blood until after the meter "beeps". Blood will automatically be drawn into the Test Strip.

6. The time begins counting down from 60 seconds. After 60 seconds the blood glucose result will appear in the Display window.

7. Using the foil packet saved in step 5, place the open flaps around the strip and remove. The meter turns off automatically.

Reporting Results

Test results are recorded on the patient's medical record by using the CH-12 form.

Procedure Notes

1. Insert the Test Strip with a firm motion until it stops completely.

2. To ensure the Test Strip fills completely, hold the Test Strip (touching the blood drop) until after the "beep".

3. Always make sure the function Number printed on the Code Strip packet and the Test Strip foil and carton matches the Function Number displayed in the Display Window.

Medical Management

Follow specific Department for Public Health Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

See operator's manual "Troubleshooting" section, page 35.

Limitations of Procedure

1. Glucometer Elite is not validated for and should not be used for testing neonatal blood specimens.

2. At normal glucose levels results are not significantly affected by hemocrits in the range of 20% to 60%. At glucose levels above 300mg/dl, hemocrit levels above 55% will cause lowered results.

3. The manufacturer states the reportable range as 40 to 500 mg/dl.

4. Ancillary blood glucose testing with any reagent test strip should not be attempted with severely ill patients, severely dehydrated patients, or patients in shock.

Instrument Maintenance

1. If the Meter exterior needs cleaning, dampen a facial tissue with fresh water and wipe the Meter and Display Window carefully. Avoid exposure to dust, cold, heat, and humidity.

2. **AVOID TOUCHING EITHER END OF THE TEST STRIP. AVOID TOUCHING THE METER END OF THE CODE STRIP OR THE CHECK STRIP. DO NOT ALLOW TEST STRIPS OR METER PORT TO BECOME WET.**

3. Avoid dropping the meter as this could result in instrument electrical malfunctions.

4. BATT will be displayed when meter batteries become weak or low. Manufacturer states meter will provide accurate results until the battery power is completely exhausted.

References

1. Glucometer Elite User Guide, Miles Inc., Elkhart, IN, 1993.

2. Glucometer Elite Blood Glucose Test Strips and Code Strip, product insert, Miles, Inc., Elkhart, IN, 1992.

3. Glucometer Elite Normal Control Solution, product insert, Miles, Inc., Elkhart, IN, 1992.

4. Bayer Corporation, 800-348-8100

Glucose
by Cholestech LDX

Principle

Glucose is an important sterol used in metabolism as a precursor of various steroid hormones. Glucose monitoring is used in the diagnosis and treatment of disorders involving excessive Glucose in blood or lipid and lipoprotein metabolism disorders. The National Institute of Health (NIH) has determined that increased levels of blood Glucose are a major cause of coronary disease. The Cholestech LDX uses a modification of the Allain method. Plasma is separated from the pre-measured heparinized whole blood by a patented filtration system built into the testing cassette.

The plasma is transferred to the reagent pads where glucose is measured by an enzymatic method that uses glucose oxidase to catlyze the oxidation of glucose to gluconolatone and hydrogen peroxide. The hydrogen peroxide oxidizes the color indicator yielding a color endpoint. The intensity of the color produced is proportional to the glucose concentration in the specimen. The end color is read by reflectance photometry using the Cholestech LDX.

Equipment and Supplies

Cholestech LDX Analyzer
Cholestech LDX Glucose Test Cassettes
Cholestech LDX Capillary Pipettor
Cholestech LDX Capillary Pipette Tubes
Cholestech LDX Controls (Level 1 & 2)
Skin-puncture blood collection supplies

Specimen Collection & Preparation

Obtain a drop of whole blood by finger puncture and fill the Cholestech LDX Capillary Pipette Tube. See Appendix B, "Specimen Collection," for detailed collection techniques.

Hemolyzed specimens are not satisfactory for testing.

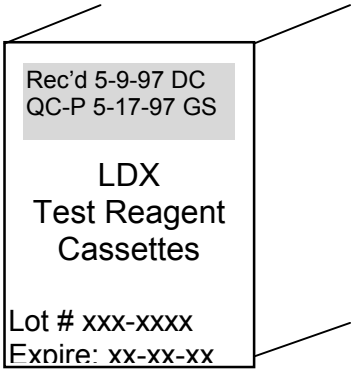
Reagent Preparation and Storage

Cholestech LDX Glucose Test Cassettes are stable until the expiration date on the pouch label, when stored in original container at 2-8°C. Allow refrigerated test pouches to reach room temperature prior to testing. Do not open the pouch until immediately before collecting the sample. Protect test cassettes magnetic strip from magnetic fields, do not touch, or allow to get wet.

Keeping Track of Reagent Shipments:

4. When a shipment of reagent cassettes are received into the laboratory mark date received and initials on each box.
5. Prior to patient testing perform and document two levels of quality control on each lot number and each shipment of reagent cassettes received. Mark each box with the following to indicate quality control has been performed and found acceptable: "QC-P", date, and initials below the date received.
6. If the cassette box you are about to open is not marked "QC-P", date, and initials you must perform and document two levels of quality control prior to performing any patient testing.

Example:



Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Cleaning the Cholestech

1. Clean the outside of the Cholestech LDX as needed. See the operator's manual for instruction.

2. Clean the cassette holder tray as needed. See the operator's manual for instruction.

Cholestech System Check

1. Cholestech analyzer automatically runs a self test upon powering up. When self test is completed the display should show "Selftest OK". Consult the operator's manual if another message appears.

2. Allow the Cholestech analyzer to warm up (approximately 5 minutes).

3. After verifying the "Selftest OK", press the RUN button. The drawer will open. The display will read "Load cassette and press RUN".

4. Place the Optics Check Cassette into the cassette holder tray and press RUN.

5. The display will show "Optics Check ##-##-##-##". Record the values and verify they are in acceptable range as printed on the Optics Check Cassette label.

Control Storage and Use

1. Perform and document two levels of quality control
- on each new shipment of cassettes,
- on each lot number of cassettes received,
- if you think cassettes may have been improperly stored,
- anytime the instrument has been moved to a new testing location, and
- anytime the test's performance is in question.

Note: See also page 3 "Keeping Track of Reagent Shipments"

2. The Cholestech controls are stable unopened and refrigerated (2-8°C) until

the expiration date listed on the box. Opened vials are stable 30 days refrigerated or at room temperature (36 - 86°C). Do not freeze. Do not use past the expiration date.

3. Allow refrigerated controls to come to room temperature (approximately 10 minutes) prior to testing.

4. Gently mix controls by inverting (at least 8 times). Use the mini-pet pipette to dispense the control material onto the test cassette in step 4 below.

5. Follow the testing procedure as written below.

6. Two levels of quality control must be within acceptable limits prior to reporting any patient test results. Consult the operator's manual for instruction when controls fail.

Test Procedure

1. Verify the daily check and quality control, as described above, have been performed and documented.

2. Remove one Glucose cassette from the foil pouch, and place it on the working surface with the print side up. Hold cassette by the short sides only. Do not touch the black reaction bar or the magnetic strip.

3. Collect the patient's specimen in the capillary tube without any air bubbles. Specimen must be used within 4 minutes of collection.

4. Dispense the sample into the sample well in the center of the cassette and load into the analyzer without delay.

5. To open the drawer press the RUN button. The instrument will perform the "Selftest" and the drawer will open.

6. Holding the cassette horizontally and not touching the magnetic strip, place the cassette into the analyzer. The magnetic strip should be on the right side.

7. Press RUN, the drawer will close and testing will begin. The display will show "Glucose Test Running".

8. The analyzer will beep to signal testing is complete and the test result will appear on the display.

9. Record the test results from the display. No calculations are required.

10. Remove the test cassette.

11. After a test has been performed. Press the RUN button twice to perform a new test.

Procedure Note

The cassette must be loaded into the analyzer for testing without delay once the specimen has been added.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

Limitations of the Procedure

1. Capillary samples must be obtained from a free-flowing puncture site. Excessive squeezing or milking may produce erroneous results.

2. Fluoride, oxalate, or citrate anticoagulants will interfere with the Glucose test and should not be used.

3. The manufacturer states Cholestech can read Glucose from 50-500 mg/dL. Results outside the stated range will result <100 or >500 mg/dL and the

patient should be re-tested by a reference laboratory with a wider testing capability.

Problem Solving

See the operator's manual "Troubleshooting" section.

References

Cholestech LDX Test Cassettes Package Insert, Cholestech Corp. Hayward, CA No date given.

Cholestech LDX Operator's Manual, Cholestech Corp. Hayward, CA No date given.

Cholestech LDX "Quality Control for Laboratory Testing", Technical Bulletin, No. 109, June 1996, Cholestech Corp. Hayward, CA.

Letter from the Cholestech Technical Services Manager (Sue Willson, MT), "Cholestech defines a run as a cassette lot", May 8, 1997.

Hematocrit

by centrifugation

Principle

The Hematocrit is a measure of the ratio of red blood cells to whole blood. This measure is performed on a small sample of capillary or venous blood and is used in the detection of anemia, blood loss, and polycythemia.

Hematocrit by centrifugation is a direct measure method using a standardized procedure. The blood is collected into two standardized capillary tubes which are centrifuged under standardized conditions to separate the red blood cells from the rest of the whole blood. The total blood column and red cell column are then measured, and the percent red blood cells is calculated.

Specimen Collection

Obtain whole blood by finger puncture. Fill two heparinized capillary tubes two-thirds full, seal one end with tube sealant, and analyze immediately. See the appendix "Specimen Collection" for detailed collection technique.

Equipment

1. Capillary tubes that meet the following specifications:
   a. Disposable
   b. Composed of borosilicate glass type I, class B or soda lime glass type I
   c. 75 ±0.5 mm in length ,internal diameter 1.07 to 1.24 mm, wall thickness
      0.18 to 0.23 mm
   d. Contain an anticoagulant (heparin)

2. Capillary Tube Sealant: a clay-like sealing compound in plastic trays 5 to 7 mm deep.

3. Microhematocrit centrifuge meeting the following specifications:
   a. Radius greater than 8 centimeters
   b. Capable of reaching maximum speed within 30 seconds
   c. Capable of sustaining a Relative Centrifugal Field (RCF*) of 10,000 to 15,000 x g for 5 minutes without exceeding 45°C.
   d. Equipped with an automatic timer calibrated at 30 second intervals from 0 to 5 minutes.

$$*RCF = (11.18 \times 10^{-6}) \times \left(\frac{\text{radius of centrifuge head}}{}\right) \times \left(\frac{\text{Centrifuge rotating speed in rpm}}{}\right)^2$$

4. Quality control materials

Reagent Storage

1. Heparinized capillary tubes are stored at room temperature in the original containers with the lid on to prevent contamination and excess moisture.

2. Do not use past the printed expiration date.

3. Tube sealant should be stored at room temperature. Tube sealant that has dried out and cracked or does not adhere well to the inner walls of the capillary tubes should be replaced.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department policies.

2. Treat all blood and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Calibration

See centrifuge maintenance section of this procedure.

Control Storage and Use

1. Perform and document two levels of quality control (high and low levels) every day of testing and whenever a new vial of capillary tubes is opened.

2. Follow manufacturer's instruction for storage and use of the control material as printed on the package insert.

3. Do not use past the printed expiration date.

4. Follow the testing procedure as written below.

Test Procedure

1. Obtain a blood specimen from a free-flowing finger- puncture site and fill two capillary tubes 2/3 full.

   Placing the gloved index finger over the dry end of the filled tube will prevent the blood from escaping from the tube.

2. Wipe off the outside of the dirty tip with tissue or gauze, if indicated, using a quick downward motion so as not to allow the blood to soak the tissue.

3. Turn the tube around and seal the dry end of the tube with the tube sealant. Hold the tube at a 90° angle to the sealant. Push the tip into the sealant while twisting the tube back and forth by rotating the thumb and index finger. Do not press hard. The sealant should enter the tube about 5 mm. If not, push the tip into the sealant a second time.

4. After sealing, the sample should be centrifuged within 5 minutes of collection. Some of the plasma may evaporate during excessive delays and cause a false high test result.

5. Place the filled tubes in the radial grooves of the microhematocrit centrifuge with the sealed end against the outer rim.

6. Record the position of the tubes in the centrifuge for proper patient identification.

7. Close the centrifuge lid and set the instrument to centrifuge for 5 minutes at 10,000 to 15,000 G.

8. When the centrifuge has come to a complete stop open the lid and observe the inside of the centrifuge for evidence of shattered or leaked tubes. If a tube is found to be less than half full, completely missing, or the plasma appears hemolyzed, the test must be repeated.

9. Determine and report the hematocrit values as determined below.

Reporting Results

1. During centrifugation, the heaviest group of cells (RBC), become packed to the bottom of the capillary tube. The next heaviest group of cells, the white blood cells (WBC) and platelets (thrombocytes), appear as a narrow white band and are packed on top of the red cells to form what is known as the buffy layer or buffy coat. The lightest material, the plasma, is above all these.

2. Using a microhematocrit reading device determine the value of the hematocrit. Align the top of the sealant/blood line at 0, and the bottom of the meniscus of the plasma at 100%. The red cell column extends from the sealant to the buffy coat, but does not include the buffy coat. Read the value of the hematocrit from the reading device. The two tubes on the same patient should agree within 2% or the test must be repeated.

Procedure Notes

1. Centrifuging the hematocrit tubes less than 5 minutes or allowing the tubes to sit more than 10 minutes before reading may cause false high test results.

2. The time and speed of centrifugation are extremely important in order to obtain accurate hematocrit test results. Using equipment that did not

meet the National Committee for Clinical Standard (NCCLS) outlined in this procedure may lead to inaccurate test results.

3. Blood must be mixed with an anticoagulant that will neither shrink nor swell the cells, nor significantly dilute the blood. Heparin or sodium ethylenediaminotetraacetate (Na_2EDTA) are the anticoagulants of choice in microhematocrit testing.

4. When blood is obtained by skin puncture, the first drop of blood is wiped away because it may contain tissue fluid. Excess pressure or squeezing should be avoided.

5. Incomplete sealing of the hematocrit tubes will give false low results, because as the tubes spin, there is a greater loss of red cells than of plasma.

6. Hematocrit by centrifugation will always include some trapped white cells, platelets and, particularly trapped plasma. The amount of trapped plasma will be increased in patients with polycythemia, iron deficiency anemia, sickle cell anemia, and some other disease processes.

7. If a hemoglobin test is also performed the correlation between hemoglobin and hematocrit can be checked. Roughly hemoglobin times three equals hematocrit. Example: Hgb of 12g/dL x 3 = ~36% Hct. This correlation may not hold true in some disease processes.

8. To verify the accuracy of the reading device compare it to a ruler with centimeter (cm) markings:
   a. Align the ruler (cm scale) next to the scale on the reader card, placing the 0 cm mark next to the 0% mark. Note where the 100% mark aligns on the ruler.
      Example: 0% aligned to 0 cm; 100% aligned to 6.5 cm.
   b. Next, calculate half of the measured length of the card scale. 6.5 cm/2 = 3.25 cm. Check that 50% on the reader card correctly aligns with the calculated value.
      Example: 50% is correctly aligned at the measured length of 3.25 cm.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Centrifuge Maintenance

Follow the maintenance schedule as specified by the centrifuge manufacturer. Below are general recommendations for those who have misplaced the manufacturer's operator's manual.

Monthly

1. Check the rubber gasket along the outer wall of the centrifuge head for wear and cracks. Replace gasket as needed, clean the centrifuge interior and exterior regularly with disinfectant soap and water.

2. Observe the area where the test samples lay for excessive heat following a daily average number of consecutive runs.

3. Observe any performance variations between runs.

Yearly

4. The microhematocrit centrifuge timer should be checked against a calibrated stop watch.

5. The centrifuge should be checked for proper calibration. Revolutions per minute (rpm) should be checked with a tachometer, strobe light, or an electronic meter.

6. A motor inspection and lubrication should be performed by a service technician as often as specified by the manufacturer and/or as needed.

References

Brown, Barbara A., Hematology: Principles and Procedures, Lea & Febiger, Philadelphia, Pa, 1980.

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

Kentucky Department for Health Services, Division of Laboratory Services past workshop materials.

National Committee for Clinical Laboratory Standards, Procedure for Determining Packed Cell Volume by the Microhematocrit Method, Volume 5 Number 5, May 1985.

National Committee for Clinical Laboratory Standards, Physician's Office Laboratory Guidelines, 1989.

Hemoglobin
by HemoCue

Principle

Hemoglobin is the major component of the red blood cells, its main function is the transport of oxygen and carbon dioxide between the lungs and tissues.

HemoCue uses a modified azidemethemoglobin reaction to measure the content of hemoglobin in whole blood. The sample is collected into a microcuvette by capillary action from a finger-puncture site. The inner walls of the cuvette reaction chamber are coated with the test reagents. The red blood cell walls are disintegrated by sodium desoxycholate, releasing the hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with azide to form azidemethemoglobin. The cuvette has been placed into the HemoCue photometer where a colorimetric reading is made and the results are displayed in g/dL.

Specimen Requirements

1. Obtain whole blood by finger puncture, fill the microcuvette, and analyze immediately. See the appendix "Specimen Collection" for detailed collection technique.

2. The cuvette should be filled in one step, never top off a cuvette.

3. Microcuvettes containing bubbles, hemolysis, or small blood clots are unsatisfactory for testing.

Equipment and Supplies

HemoCue hemoglobin photometer
HemoCue control cuvette
HemoCue microcuvettes
Two levels of quality control material
Specimen collection supplies

Reagent Storage

1. HemoCue microcuvettes are stored at room temperature.

2. Microcuvettes must be used prior to the printed expiration date or three months after the initial date opened, whichever is earlier.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood and used testing apparatus as potentially infectious waste. Follow your health department disposal policies.

Calibration

A calibration verification check should be performed and documented each day of testing with the "HemoCue Red Control Cuvette". A HemoCue that fails to give a calibration check within acceptable limits should not be used until the problem is corrected, use a back up test method as needed.

Calibration verification procedure:

1. Turn the HemoCue "on". Display should read "Hb".

2. Pull the cuvette holder out to the insertion position. Wait about 6 seconds.
   Display should read "Ready" with three flashing dashes.
3. Place the red control cuvette into the holder and push it in completely. Display should read "measuring" with three flashing dashes.

4. Wait about 12 seconds and a reading should appear. This reading must agree with the assigned value (____± 0.3 g/dL) to insure proper optical function. Document the calibration check results on the quality control record.

5. If the calibration procedure fails, verify that the optical viewing path is clean--this is the most common reason for calibration check failure. Consult the maintenance section for instruction. If the problem persists after cleaning, call HemoCue technical services for further assistance.

Control Storage and Use

To assure proper function of the entire test system two levels of external quality control must be performed and documented each day of testing.

1. Perform and document two levels of external quality control (high and low levels) every day of testing and whenever a new vial of microcuvettes is opened.

2. Follow manufacturer's instruction for storage and use of the control material as printed on the package insert.

Note: Control materials contain stabilizing additives and react more slowly than fresh whole blood specimens when tested by the HemoCue test method. Therefore, the manufacturer of the HemoCue recommends that the control material be allowed to set in the cuvette for at least 60 seconds prior to testing.

3. Do not use control materials past the printed expiration date.

4. Follow the testing procedure as written below.

5. Two levels of quality control must be within acceptable limits prior to reporting any patient test results.

Testing Procedure

1. Verify the daily quality control and calibration have been performed and documented.

2. Remove a cuvette and immediately recap the vial to keep out moisture. Avoid touching the sample eye of the cuvette; grasp by the winged, frosted end. The cuvette must remain clean and scratch free for accurate test results.

3. Obtain a drop of whole blood, not the first drop, and fill the microcuvette. Avoid bubbles which cause the sample to be unsatisfactory for testing.

4. Wipe any excess blood from the outside of the cuvette, using a lint free and clean wipe material.

5. The filled cuvette should be analyzed immediately and, at the latest, 10 minutes after it has been filled.

6. Place the filled cuvette into the black slide holder. The display should show "Ready". Insert the slide holder into the instrument. Wait 30 - 50 seconds, the display will show the test results in g/dL.

7. After 5 minutes the display will return to "Ready".

8. A re-measurement may be initiated, as long as the 10 minute reaction stability has not elapsed. Place the slide holder in the outer position, wait for the display to show "Ready". Push the cuvette into the analyzer and wait about 20 seconds for the display to show the results.

9. Discard the used microcuvette as medical waste following appropriate health department policies.

Reporting Results

Test results are recorded on the patient's medical record using the CH-12 form.

Procedure Notes

1. Failing to wipe the alcohol from the puncture site before the stick, may cause hemolysis of the specimen. Hemolyzed specimens can not be detected by the instrument and are unsatisfactory for testing.

2. The first drop of blood from the skin puncture may contain tissue fluid, diluting the test sample and causing inaccurate test results. Therefore, it is necessary to wipe away the first drop of blood and collect the next drop of blood in the microcuvette for testing.

3. Excessive squeezing during specimen collection may cause the specimen to be diluted with tissue fluids and/or result in specimen clotting.

4. Keep the cuvette slide holder clean to insure accurate results. The photometer cannot read properly when its light path is blocked by debris.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

The HemoCue does have built in error codes (900-905) that may display when a problem has developed.

See the operator's manual "Troubleshooting Guide" for assistance.

Maintenance

1. Each HemoCue has an assigned red control cuvette. Health departments owning more than one HemoCue must verify by a serial number check that the red control cuvettes are matched to the correct HemoCue for accurate calibration checks.

2. The red control cuvette must be free of fingerprints, dirt, scratches, and cracks to perform accurately.

3. The red cuvette may be cleaned with non-abrasive tissue such as lens paper. When necessary, soap and water may be used, then rinse well and dry completely with non-abrasive tissue.

4. Red cuvettes that become lost or permanently damaged must be replaced. Contact the manufacturer for details (800-323-1674).

5. The black cuvette slide holder must be clean and the small lens window unobstructed. The black cuvette slide holder can be cleaned with mild soap and water or 1:10 bleach solution, rinsed well, and dried completely before placing back into the instrument.

6. If the red cuvette values are still out of range following the above cleaning steps, call HemoCue technical services for further instruction (800-323-1674).

7. Some of the early HemoCue models had rechargeable batteries. These batteries must be replaced after several years of use. Alternatively, the HemoCue electrical adapter can be used without any batteries in the unit. When a "low batt" code appears on the screen the batteries should be replaced or removed. Failure to remove the old batteries may effect the electronics of the HemoCue.

8. The exterior of the photometer should be cleaned as necessary with a mild soap solution.

9. The manufacturer states the HemoCue readable range as 0 - 18.0 ± 2% and 18.0-25.6 ± 4%.

References

Brown, Barbara A., Hematology: Principles and Procedures, Lea & Febiger, Philadelphia, Pa. , 1980.

HemoCue Quality Assurance Program Manual, 3rd Edition, November, 1990.

HemoCue Operator's Manual,, HemoCue Inc., Angelholm, Sweden,

HemoCue Microcuvette package insert, no date given.

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

Stanbio QuPID Plus One-Step
Serum/Urine Pregnancy Test

Principle

Human chorionic gonadotropin (hCG) is a hormone secreted by the developing placenta shortly after fertilization. During normal pregnancy, hCG can be detected as early as 6 days following conception, doubling every 1.3 to 2 days. The detection of hCG is an excellent marker for confirming pregnancy. The Stanbio QuPID Plus test detects the intact hCG molecule in serum or urine.

The Stanbio QuPID Plus test is a qualitative immunoassay for the detection of human chorionic gonadotropin (hCG) in serum or urine. The test utilizes a combination of monoclonal and polyclonal antibody reagents to selectively detect elevated levels of hCG. The assay is conducted by the addition of specimen into the sample well and observing for the formation of colored lines in the result area. The specimen migrates via capillary action along the membrane and reacts with the antibody-dye conjugate. Positive hCG specimens react with the specific antibody-hCG-colored conjugate and form a colored line in the Specimen Zone (S) portion of the membrane. Absence of this colored line suggests a negative result. To serve as a positive procedural control, a colored line in the Control Zone (C) will always appear regardless of the presence or absence of hCG.

Specimen Collection Serum

1. Specimens should be collected under standard laboratory conditions; avoid hemolysis.

2. Specimens can be stored in the refrigerator up to 48 hours, if testing must be delayed. Refrigerated specimens should be allowed to come to room temperature prior to testing for reliable results.

Specimen Collection Urine

1. The first early morning urine specimen is the best specimen, because it generally contains the most hCG. Any urine specimen is suitable for testing; however, if a urine is too diluted (has a very low specific gravity) it may not contain enough hCG to give a positive test.

2. The specimen should be collected in a clean, dry plastic or glass container. See the appendix for specimen collection techniques.

3. Specimens can be stored in the refrigerator up to 72 hours, if testing must be delayed. Refrigerated specimens should be allowed to come to room temperature prior to testing for reliable results.

Equipment

Sealed foil pouch containing:

One Test Device *Do not use test devices which have become wet or which have been left out of the foil pouch.*
One Disposable specimen dropper
A dry flat working surface
Quality control material (available from manufacturer)
Timer with audible beep, accurate at 3 and 5 minute intervals

Kit Use and Storage Instructions

6. Each QuPID Plus test is stable until the expiration date on the foil pouch when stored at room temperature (59-86°F/15-30°C).

7. Do not use test devices which have become wet or which have been left out of the foil pouch.

Quality Control

1. External Controls:

Perform and document two levels of external quality control (minimal positive and negative) every day of testing and when a new kit is opened. Two levels of quality control must be within acceptable limits and documented for each day of testing and each test kit opened prior to reporting any patient testing.

2. Internal Controls:

A positive procedural control (Control Zone "C") is built into the QuPID Plus test device. This control line will always appear if the test is performed correctly and if the device is working properly. An absence of this control line indicates incorrect procedure or deterioration of reagents.

The absence of interfering background is a negative procedural control. If background color appears in the result area which interferes with the ability to read the test results, the result may be invalid.

3. If the control line fails to appear with a repeat assay, do not report patient results. Contact Stanbio Technical Service: 800/531-5535 or 210/222-2108.

4. Use external controls with a serum based when performing serum tests on patients. Store controls according to the manufacturer's instruction. Do not use past the expiration date. Do not use if contamination is evident.

5. Allow controls to warm to room temperature prior to testing.

6. Do not mix caps from different control vials. (For example: To avoid mixing the vial caps, open control vials one at a time, replacing each top before opening another vial.)

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Testing Procedure

1. Verify the control has been performed and documented.

2. Specimens should be at room temperature prior to testing.

3. Remove the QuPID Plus test device from the protective pouch and place it on a flat, dry surface.

4. Label the device with patient or control identifications.

5. Holding the disposable specimen dropper vertically, dispense 2 full drops (approximately 0.120 mL) of specimen into the round sample well. See the package insert for an illustration.

6. Set the timer for **Serum** (5) minutes **Urine** (3) three minutes and read the test promptly at the specified time.

THE TEST CASSETTE SHOULD NOT BE MOVED OR TILTED UNTIL THE ASSAY IS COMPLETE.

Expected Results (See package insert for illustration)

Healthy men and non-pregnant women do not have hCG levels detectable by the Stanbio QuPID Plus Test. In normal pregnancy, levels of 20 mIU/mL hCG can be reached 2-3 days before the first missed menstrual period. HCG levels peak about 8 weeks after the last menstrual period and then decline to lower values during the remainder of the pregnancy. Following delivery, hCG levels rapidly decrease and usually return to normal within days after parturition.

Negative test: the test is negative when only one line appears in the results area at the Control Zone (C).

Positive test: the test is positive when two colored lines appear in the results area, one at the Control Zone (C), and one at the Specimen Zone (S). A weak positive result may show a lighter colored line in the Specimen Zone (S).

Invalid Results: the test is invalid if no line appears at the Control Zone (C) even if a colored line appears at the Specimen Zone (S). In this case, the test should be repeated.

NOTE: Also read procedure notes section.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

A specimen with a low level of hCG may show some color development over time. It is very important to read the test at the time specified and not later.

It is a good laboratory practice to resample and retest patients yielding weak positive results after an additional 48-72 hours. Running external controls near the 20 mIU/mL cut-off may guide the interpretation of weak positive results.

Do not use test devices which have become wet or which have been left out of the foil pouch.

Limitations of the Procedure

1. The QuPID Plus test kit is for the qualitative detection of hCG in serum or urine. The manufacturer states this kit has a sensitivity of 20 mIU/mL. This level of hCG has been observed as early as 2-3 days before the first missed menstrual period. No interfering substances have been identified, the tested substances are listed in the manufacturer's package insert.

2. High urine hCG levels may occur in patients suffering from chorionic epithelioma or hydatid mole. In these cases a false positive may occur.

3. Normal pregnancy cannot be distinguished from an ectopic pregnancy base on hCG levels alone. Spontaneous miscarriage may also cause confusion in interpreting assay results.

4. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). These specimens may demonstrate either false positive or false negative results when tested with assays which employ mouse monoclonal antibodies.

5. HCG levels may remain detectable for several weeks after normal delivery, delivery by caesarean section, spontaneous abortion, therapeutic abortion or hCG injections.

6. Positive results from early pregnancy may later prove negative due to natural termination of the pregnancy. This is estimated to occur in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall. It is therefore recommended when using a sensitive hCG assay such as the QuPID Plus, that weak positive results be re-tested 48-72 hours later.

7. If a urine specimen is too dilute (i.e., low specific gravity) it may not contain representative levels of hCG. If pregnancy is still suspected, a first morning specimen should be obtained 48-72 hours later and re-tested.

8. As with all pregnancy tests, the final diagnosis should be based on a correlation of test results with typical clinical signs and symptoms. If the qualitative interpretation is inconsistent with the clinical evidence, results should be confirmed by an alternate hCG method.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department at 800/531-5535 or 210/222-2108.

References

Stanbio QuPID Plus One-Step Pregnancy Test Procedure No. 1230. 3/99

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

Serum Pregnancy Test
Confirms hCG Serum/Urine

Principle

Human chorionic gonadotropin (hCG) is a hormone secreted by the developing placenta shortly after fertilization. The hCG hormone is released into the maternal serum and subsequently excreted in the urine. The appearance of hCG soon after conception and its subsequent rise in concentration during early gestational growth make it an excellent marker for the early detection of pregnancy. A number of conditions other than pregnancy including hydatidiform mole and associated neoplastic diseases can cause elevated hCG levels comparable to those observed in early pregnancy. These diagnoses should be considered if appropriate to the clinical evidence.

Mainline Confirms hCG Serum/Urine test is a chromatographic immunoassay for the rapid qualitative determination of hCG in serum or urine. The membrane is pre-coated with anti-alpha hCG capture antibody. When hCG is present in the patient sample it reacts with the colloidal gold particles coated with anti-beta hCG monoclonal antibody producing a visible color band.

Specimen Collection

SERUM:

1. Collect a red stoppered tube of whole blood.

2. Process the whole blood to obtain serum.

(See the appendix for specimen collection and processing techniques).

Grossly hemolyzed specimens are unsatisfactory for testing.

URINE:

1. The first early morning urine specimen is the best specimen, because it is the most concentrated. Any urine specimen is suitable for testing; however, if a urine is too dilute (has a very low specific gravity) it may not contain representative levels of hCG.
2. The specimen should be collected in a clean, dry plastic or glass container. See the appendix for specimen collection techniques.

3. Specimens can be stored in the refrigerator up to 72 hours, if testing must be delayed. Refrigerated specimens should be allowed to come to room temperature prior to

testing for reliable results.

4. Urine samples exhibiting visible precipitates should be filtered, centrifuged or allowed to settle and clear aliquots obtained for testing.

Equipment

Confirm hCG Test Kit (test device and transfer pipette)
Quality control material (available from manufacturer)
Timer accurate at 5 minutes (manufacturer will supply upon request)

Kit Use and Storage Instructions

1. Confirms hCG serum/urine test kits may be stored at refrigerated or room temperature (2-30°C). Allow refrigerated test devices to reach room temperature prior to testing.

2. Do not use kits past the expiration date.

Quality Control

1. Perform and document two levels of external quality control (low level positive and negative) each day of testing, whenever a new kit is opened, and anytime a test's performance is in question.

2. External urine controls should be stored, prepared, and used according to the manufacturer's instruction. Do not use past the printed expiration date. Follow the test procedure as written on the next page.

3. Two levels of quality control must be within acceptable limits on the day of test, prior to any patient testing.

4. Built-in procedural controls must be observed and documented with each test run. The built in procedural controls appear as follows:

- A pink colored band must always appear (positive control) at the Control Region (C). Absence of this band indicates the test is invalid. *(Pink indicates the test cassette functioned properly)*

- The background in the test result window will clear from a deep pink to a white or pale pink (negative control) providing a distinct result. If the background does not clear as described the test is invalid. *(A clear background indicates no interfering substances were present)*

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Testing Procedure

1. After the test device has come to room temperature, remove it from the pouch, and label the device with patient or control identification.

2. Holding the pipette in a vertical position, dispense 5 drops of specimen into the sample well. Use a separate pipette and testing device for each specimen.

3. Set a timer for 5 minutes and wait for colored bands to appear. Positive results may be observed as quickly as 40 seconds. However, a 5 minute reaction time is required before reporting a negative result. Results must be interpreted at 5 minutes and not thereafter.

Expected Results

Each testing device includes a control (C) and a reference (❖) band. The control band will produce a colored band to indicate proper technique and reactive reagents. No test shall be reported if the control band is not present when the test is read. The reference band is equivalent to 25 mIU/mL (low positive) and is present for comparison. Any shade of pink is a valid color band. See color graphic in package insert.

Positive test: Three distinct colored bands are visible, the (C) control band, the (❖) reference band, and (T) test band.

Borderline test: Results are not conclusive and should be retested in 48-72 hours Three colored bands are visible, the (C) control band and (❖) reference band are distinct, but the (T) test band has less color and is less distinct than the reference band. See color graphic in package insert.

Negative test: After 5 minutes reaction time the (C) control and (❖) reference bands are visible with no band in the (T) test region.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form**.**

Procedure Notes

1. In normal pregnancy hCG can be detected as early as 7 days following conception. The hCG levels rise rapidly and peak about 10-12 weeks into the pregnancy.

2. The test results should not be interpreted beyond 5 minutes because non-specific color may develop over extended time, which could be misinterpreted as a weak positive result.

3. No color bands on the test area denotes an invalid test, caused by improper test procedure or deterioration of reagents. The test must be repeated using a new test device and pipette.

4. The shade of pink on the test band will vary depending on the concentration on hCG present. However, the color variation is not an accurate means of hCG quantitation.

5. Weakly reactive positive or negative test results in patients suspected to be pregnant should be confirmed by retesting a fresh early morning sample obtained 48-72 hours later, or by a quantitative hCG assay.

Limitations of the Procedure

1. Sensitivity of this kit is stated as 20 mIU/mL or greater.

2. No interfering substances have been identified; the tested substances are listed in the manufacturer's package insert.

3. A number of conditions other than pregnancy, including trophoblastic disease, may cause elevated levels of hCG. These diagnoses should be considered if appropriate to the clinical evidence.

4. Natural termination occurs in 22% of clinically unrecognized pregnancies. A borderline test could be due to recent natural termination of the pregnancy. Therefore it is good laboratory practice to retest borderline test results in an additional 48-72 hours.

5. If a urine specimen is too dilute it may not contain enough hCG to be detected, when this is suspected retest (48-72 hours later) with a first morning specimen.

6. In normal pregnancy, hCG can be detected in serum as soon as 7 days after conception, doubling every 1.3 to 2 days. At the time of the first missed menstrual period, hCG concentration is about 100 mIU/mL, and peak levels of 30,000 to 200,000 mIU/mL are seen at the end of the first trimester.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/ Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department.

References

Mainline Confirms hCG Serum/Urine - package insert, 4/96.

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

Stanbio QuPID One-Step
Urine Pregnancy Test

Principle

Human chorionic gonadotropin (hCG) is a hormone secreted by the developing placenta shortly after fertilization. During normal pregnancy, hCG can be detected as early as 6 days following conception, doubling every 1.3 to 2 days. The detection of hCG is an excellent marker for confirming pregnancy. The Stanbio QuPID test detects the intact hCG molecule in urine.

The Stanbio QuPID test is a qualitative immunoassay for the detection of human chorionic gonadotropin (hCG) in urine. The test utilizes a combination of monoclonal and polyclonal antibody reagents to selectively detect elevated levels of hCG in urine. The assay is conducted by the addition of urine specimen into the sample well and observing for the formation of colored lines in the result area. The urine specimen migrates via capillary action along the membrane and reacts with the antibody-dye conjugate. Positive hCG specimens react with the specific antibody-hCG-colored conjugate and form a colored line in the Specimen Zone (S) portion of the membrane. Absence of this colored line suggests a negative result. To serve as a positive procedural control, a colored line in the Control Zone (C) will always appear regardless of the presence or absence of hCG.

Specimen Collection

1. The first early morning urine specimen is the best specimen, because it generally contains the most hCG. Any urine specimen is suitable for testing; however, if a urine is too diluted (has a very low specific gravity) it may not contain enough hCG to give a positive test.

2. The specimen should be collected in a clean, dry plastic or glass container. See the appendix for specimen collection techniques.

3. Specimens can be stored in the refrigerator up to 72 hours, if testing must be delayed. Refrigerated specimens should be allowed to come to room temperature prior to testing for reliable results.

Equipment

Sealed foil pouch containing:
- One Test Device *Do not use test devices which have become wet or which have been left out of the foil pouch.*
- One Disposable specimen dropper

A dry flat working surface
Quality control material (available from manufacturer)
Timer with audible beep, accurate at 3 minutes

Kit Use and Storage Instructions

8. Each QuPID test is stable until the expiration date on the foil pouch when stored at room temperature (59-86°F/15-30°C).

9. Do not use test devices which have become wet or which have been left out of the foil pouch.

Quality Control

7. External Controls:

Perform and document two levels of external quality control (minimal positive and negative) whenever a new kit is opened and anytime a test's performance is in question. Mark the kit with "QC-P", date, and initials to donote the kit has been quality control tested and found acceptable for patient testing. Two levels of quality control must be within acceptable limits and documented for each test kit prior to reporting any patient testing.

8. Internal Controls:

A positive procedural control (Control Zone "C") is built into the QuPID test device. This control line will always appear if the test is performed correctly and if the device is working properly. An absence of this control line indicates incorrect procedure or deterioration of reagents.

The absence of interfering background is a negative procedural control. If background color appears in the result area which interferes with the ability to read the test results, the result may be invalid.

9. If the control line fails to appear with a repeat assay, do not report patient results. Contact Stanbio Technical Service: 800/531-5535 or 210/222-2108.

10. External (urine) controls should be stored according to the manufacturer's instruction. Do not use past the expiration date. Do not use if contamination is evident.

11. Allow controls to warm to room temperature prior to testing.

12. Do not mix caps from different vials. (For example: To avoid mixing the vial caps, open control vials one at a time, replacing each top before opening another vial.)

Disposal of Hazards

3. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

4. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Testing Procedure

7. Verify the control has been performed and documented.

8. Specimens should be at room temperature prior to testing.

9. Remove the QuPID test device from the protective pouch and place it on a flat, dry surface.

10. Label the device with patient or control identifications.

11. Holding the disposable specimen dropper vertically, dispense 2 full drops (approximately 0.120 mL) of specimen into the round sample well. See the package insert for an illustration.

12. Set the time for (3) three minutes. Read the test promptly at three minutes.

THE TEST CASSETTE SHOULD NOT BE MOVED OR TILTED UNTIL THE ASSAY IS COMPLETE.

Expected Results (See package insert for illustration)

Healthy men and non-pregnant women do not have hCG levels detectable by the Stanbio QuPID Test. In normal pregnancy, levels of 20 mIU/mL hCG can be reached 2-3 days before the first missed menstrual period. HCG levels peak about 8 weeks after the last menstrual period and then decline to lower values during the remainder of the pregnancy. Following delivery, hCG levels rapidly decrease and usually return to normal within days after parturition.

Negative test: the test is negative when only one line appears in the results area at the Control Zone (C).

Positive test: the test is positive when two colored lines appear in the results area, one at the Control Zone (C), and one at the Specimen Zone (S). A weak positive result may show a lighter colored line in the Specimen Zone (S).

Invalid Results: the test is invalid if no line appears at the Control Zone (C) even if a colored line appears at the Specimen Zone (S). In this case, the test should be repeated.

NOTE: Also read procedure notes section.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

A specimen with a low level of hCG may show some color development over time. It is very important to read the test at three minutes and not later.

It is a good laboratory practice to resample and retest patients yielding weak positive results after an additional 48-72 hours. Running external controls

near the 20 mIU/mL cut-off may guide the interpretation of weak positive results.

Do not use test devices which have become wet or which have been left out of the foil pouch.

Limitations of the Procedure

9. The QuPID test kit is the qualitative detection of hCG in urine, only. The manufacturer states this kit has a sensitivity of 20 mIU/mL. This level of hCG has been observed as early as 2-3 days before the first missed menstrual period. No interfering substances have been identified, the tested substances are listed in the manufacturer's package insert.

10. High urine hCG levels may occur in patients suffering from chorionic epithelioma of hydatid mole. In these cases a false positive may occur. These diagnosis should be considered if appropriate to clinical evidence.

11. Normal pregnancy cannot be distinguished from an ectopic pregnancy base on hCG levels alone. Spontaneous miscarriage may also cause confusion in interpreting assay results.

12. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). These specimens may demonstrate either false positive or false negative results when tested with assays which employ mouse monoclonal antibodies.

13. HCG levels may remain detectable for several weeks after normal delivery, delivery by caesarean section, spontaneous abortion, therapeutic abortion or hCG injections.

14. Positive results from early pregnancy may later prove negative due to natural termination of the pregnancy. This is estimated to occur in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall. It is therefore recommended when using a sensitive hCG assay such as the QuPID, that weak positive results be re-tested 48-72 hours later.

15. If a urine specimen is too dilute (i.e., low specific gravity) it may not contain representative levels of hCG. If pregnancy is still suspected, a first morning specimen should be obtained 48-72 hours later and re-tested.

16. As with all pregnancy tests, the final diagnosis should be based on a correlation of test results with typical clinical signs and symptoms. If the qualitative interpretation is inconsistent with the clinical evidence, results should be confirmed by an alternate hCG method.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department at 800/531-5535 or 210/222-2108.

References

Stanbio QuPID One-Step Pregnancy Test Procedure No. 1220. 8/98

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

Urine Pregnancy Test
Confirm hCG

Principle

Human chorionic gonadotropin (hCG) is a hormone secreted by the developing placenta shortly after fertilization. The appearance of hCG soon after conception and its subsequent rise in concentration during early gestational growth make it an excellent marker for the early detection of pregnancy. However, other disease conditions, such as hydatidiform mole and associated neoplastic diseases, can cause hCG levels comparable to those observed in early pregnancy. Such disease conditions should be ruled out before a positive hCG result is considered diagnostic for pregnancy.

Mainline Technology Confirm hCG is a qualitative, chromatographic immunoassay for the determination of hCG in urine. The membrane is pre-coated with anti-alpha hCG capture antibody. When hCG is present in a patient sample it reacts with the colloidal gold particles coated with anti-beta hCG monoclonal antibody, producing a visible color band.

Specimen Collection

1. The first early morning urine specimen is the best specimen, because it is the most concentrated. Any urine specimen is suitable for testing. However, if a urine is too dilute (has a very low specific gravity) it may not contain representative levels of hCG.

2. The specimen should be collected in a clean, dry plastic or glass container. See Appendix B for specimen collection techniques.

3. Specimens can be stored in the refrigerator up to 72 hours if testing must be delayed. Refrigerated specimens should be allowed to come to room temperature prior to testing for reliable results.

4. Urine samples exhibiting visible precipitates should be filtered, centrifuged or allowed to settle, and clear aliquots obtained for testing.

Equipment

Confirm hCG Test Kit (test device and transfer pipette)
Quality control material (available from manufacturer)
Timer accurate at four minutes, with bell/alarm

Kit Use and Storage Instructions

1. Confirm hCG test kits may be stored at refrigerated or room temperature (2-30°C). Allow refrigerated test devices to reach room temperature prior to testing.

2. Do not use kits past the expiration date.

Control Storage and Use

1. Perform and document two levels of external quality control (positive and negative) whenever a new kit is opened and anytime a test's performance is in question.

2. Urine controls should be stored, prepared, and used according to the manufacturer's instruction.

3. Do not use past the printed expiration date.

4. Follow the test procedure as written on the next page.

5. Two levels of quality control must be within acceptable limits prior to use of the kit for patient testing.

6. Each test includes a procedural control band which will produce a colored band to indicate proper performance and reactive reagents. No test shall be reported if the control band is not present when the test is read.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all blood, body fluids, and used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Testing Procedure

1. After the test device has come to room temperature, remove it from the pouch, and label the device with patient or control identification.

2. Holding the pipette in a vertical position, dispense 5 drops of specimen into the sample well. Use a separate pipette and testing device for each specimen.

3. Set a timer for 4 minutes and wait for colored bands to appear. Positive results may be observed as quickly as 40 seconds. However, a 4 minute reaction time is required

before reporting a negative result. Results must be interpreted at 4 minutes and not thereafter.

Expected Results

The test zone contains two bands, (C) the control band and (T) the test band. Color bands may be any shade of pink.

Negative test: After 4 minutes reaction time only the (C) control band is visible.

Positive test: Two distinct colored bands are visible, the (C) control band and (T) test band.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

1. In normal pregnancy, hCG can be detected as early as 7 days following conception. The hCG levels rise rapidly and peak about 10-12 weeks into the pregnancy.

2. The test results should not be interpreted beyond 4 minutes because non-specific color may develop over extended time which could be misinterpreted as a weak positive result.

3. No color bands on the test area denotes an invalid test, caused by improper test procedure or deterioration of reagents. The test must be repeated using a new test device and pipette.

4. The shade of pink on the test band will vary depending on the concentration of hCG present. However, the color variation is not an accurate means of hCG quantitation.

5. Weakly reactive positive or negative test results in patients suspected to be pregnant should be confirmed by retesting a fresh early morning sample obtained 48-72 hours later, or by a quantitative hCG assay.

Limitations of the Procedure

1. Sensitivity of this kit is stated as 20 mIU/mL or greater.

2. No interfering substances have been identified, the tested substances are listed in the manufacturer's package insert.

3. A number of conditions other than pregnancy, including trophoblastic disease, may cause elevated levels of hCG. These diagnoses should be considered if appropriate to the clinical evidence.

4. The incidence of early loss of pregnancy has been documented to be as high as 31%. A weak positive could be due to recent natural termination of the pregnancy. Therefore it is good laboratory practice to retest weak positive results after 48-72 hours.

5. If a urine specimen is too dilute it may not contain enough hCG to be detected. When this is suspected, retest (48-72 hours later) with a first morning specimen.

6. Urine hCG levels in pregnant women begin to rise within nine to 12 days after ovulation and reach levels of up to 150,000 mIU/mL eight to 10 weeks after the last menstrual period.

Management Guidelines

Follow specific Department for Health Services Clinical Services Standards/ Guidelines for Medical Management.

Problem Solving

Consult the manufacturer's package insert or call the technical services department.

References

Mainline Confirms hCG In Urine - package insert, 9/93.

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

Group A Strep Test
by **Quidel QuickVue In-line One-step Strep A Test**

Principle

Group A Streptococcus is a bacteria responsible for about 19 % of upper respiratory infections. Streptococcal pharyngitis is more prevalent in the winter and early spring and among crowded populations, such as military bases and school aged children.

The Quidel Quick Vue in-line one-step Strep A Test is a rapid qualitative test to detect Group A Streptococcus antigen directly from patient throat swab specimens by a lateral flow immunoassay test method. A throat swab specimen is collected and placed into the swab chamber. An extraction solution is added which causes the release of specific Group A Streptococcus antigens. The extracted sample migrates by capillary action through a label pad consisting of a pink label containing rabbit polyclonal anti-strep A antibody and a blue control label. If the extracted solution contains strep A antigen, the antigen will bind to the antibody on the pink test label which, in turn, will bind with a second rabbit polyclonal anti-strep A antibody spotted on the membrane, resulting in the formation of a pink test line. A blue control line will also appear indicating the extraction reagent and test cassette functioned properly.

Specimen Collection and Preparation

Using the swabs provided in the test kit obtain the specimen from the throat, be sure to swab any white patches in the tonsillar area. (See appendix for specimen collection techniques)
Specimen swabs sealed in sterile tubes may be held at room temperature for up to 4 hours and refrigerated for up to 24 hours, if testing must be delayed.

Equipment and Supplies

Quidel In-line One-step Strep A Kit
A dry, flat working surface.
Laboratory timer with one minute intervals

Kit Use and Storage Instructions

1. Follow the expiration date printed on the kit, store at room temperature, avoid direct sunlight, and do not freeze. Kit contents are stable until the expiration date printed on the outer box.

2. The extraction reagent is acidic and can irritate the skin. Avoid contact with eyes.

Control Use

1. Perform and document two levels of external quality control* whenever a new kit is opened and with each new operator.

* A set of positive (labeled pink) and negative (labeled blue) swabs come in each test kit. Additional swabs may be requested from the manufacturer. Liquid controls are also available from the manufacturer (cat # 0354).

2. Built-in control features must be observed and documented with the first test of the day. Built-in control checks include:
   - Extraction solution turns from clear to green when ampule is broken and properly mixed. *(Indicates the solution was properly prepared)*
   - At the completion of the test (5 mins), a blue control line is visible at the letter "C" on the test cassette. *(Indicates the test cassette functioned properly)*
   - At the completion of the test (5 mins), the test window background is clear-to-light pink and uniform in appearance. (*Indicates no interfering substances were present)*

3. If controls do not perform as expected, test results are invalid and must not be reported.

Test Procedure

1. Verify the quality control, as described above, has been performed and documented.

2. Open the foil package and place testing cassette(s) on a clean, dry, level surface.

3. Gently insert the specimen swab completely into the chamber, resting the shaft on the notch at the back of the swab chamber.

4. Immediately before use, carefully crush the glass ampule inside the extraction solution bottle. Invert and shake vigorously five times to mix the solution well. Green extraction solution must be used immediately.

5. Remove the cap and invert the extraction solution bottle. Holding bottle vertically straight (not at an angle) about an inch above the specimen swab, quickly fill the chamber to the rim (approximately 10 drops).

6. Set the timer for 5 (five) minutes.

7. If the liquid has not moved across the results window in 1 minute, completely remove the swab and re-insert. If liquid still does not move across, retest with a new specimen, test cassette, and extraction solution bottle.

THE TEST CASSETTE SHOULD NOT BE MOVED OR TILTED UNTIL THE ASSAY IS COMPLETE.

8. Read results at 5 (five) minutes.

Expected Results

Positive: a pink-to-purple line next to the letter "T" and a blue line next to the letter "C" in the results window.

Negative: only a blue line next to the letter "C" in the results window.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Limitations of the Procedure

1. The Quick Vue In-line One-step strep test is designed for throat swab specimens only. Tests performed on swabs from other body sites are not valid.

2. This test will detect both viable and non-viable organisms. Therefore patients that have received treatment prior to testing could have a positive direct antigen test, yet test negative by the standard culture method.

3. This test will not differentiate asymptomatic carriers of Group A Streptococcus from those exhibiting streptococcal infections.

4. The throat swab specimen must contain ≥ 500,000 organisms of group A Strep per test in order to obtain a positive test result. Proper specimen collection is imperative to valid test results.

5.In rare cases, test specimens heavily colonized with Stapphylococcus aureus ($>10^{10}$) can yield false positive result.

6. Diagnosis should be based on the total patient picture and not on one laboratory test alone. If a false negative test result is suspected follow-up with additional testing by culture method is recommended.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable

health department disposal policies.

2. Treat all used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

References

Quidel QuickVue In-line One-step Strep A package insert, 11/96.

Balows, Albert, et. al., Manual Of Clinical Microbiology, American Society for Microbiology, Washington D.C., 5th ed., 1991.

Group A Strep Test
By Signify Strep A Test

Principle

Group A Streptococcus is a bacteria responsible for about 19% of upper respiratory infections. Streptococcal pharyngitis is prevalent in the winter and early spring and among crowded populations, such as military bases and school aged children.

The Signify Strep A Test uses color immunochromatographic technology with rabbit antibodies coated on the nitrocellulose membrane. In the test procedure, a throat swab is subjected to a chemical extraction of a carbohydrate antigen unique to Group A Streptococcus. The LabStrip is then placed in the extraction mixture and the mixture migrates along the membrane. If Group A Streptococcus is present in the sample, it will form a complex with the anti-group A Streptococcus antibody conjugated color particles. The complex will then be bound by the anti-Group A Streptococcus capture antibody and a visible blue Test Line will appear to indicate a positive result.

Specimen Collection

Collect specimens with a sterile swab from the tonsils and/or the back of the throat. Take care to avoid the teeth, gums, tongue or cheek surfaces. (See appendix for specimen collection techniques).

Equipment and Supplies

Signify Strep A Test
A dry, flat working surface
A timer or watch

Kit Use and Storage

Store LabStrips and reagents tightly capped at 15°-30°C (59°-86°F). Do not use LabStrips or reagents after expiration date.

Quality Control

Internal Procedure Controls

The Signify Strep A Test provides three levels of procedural controls with each test run. For daily quality control, Abbott Laboratories recommends documenting these controls on each day of testing:

- The color of the liquid changes from pink to light yellow as you add Extraction Reagent 2 to Extraction Reagent 1. This is an internal extraction reagent control. The color change means that you mixed the extraction reagents properly. The color change also means that the reagents are functioning properly.
- The red Control Line is an internal control. The LabStrip must absorb the proper amount of sample and the LabStrip must be working properly for the red Control Line to appear. For the LabStrip to be working properly, the capillary flow must occur.
- A clean background is an internal background negative control. If no interfering substances are in the specimen and the LabStrip is working properly, the background in the Control Line area will clear. A discernible result will be seen.

If the red Control Line does not appear, the test may be invalid. If the background does not clear and interferes with the test result, the test may be invalid. Call Abbott Laboratories Customer Support Center if you experience either of these problems.

External Quality Control Testing

Each kit contains Positive and Negative Control materiel. The Controls are for external quality control testing. Use the Controls to test that the extraction reagents and the LabStrips are working. Also use the Controls to test that you are able to correctly perform the test procedures. Some commercial controls may contain interfering additives. Therefore it is recommended that you do not use other commercial controls with the Signify Strep A Test.

In addition to standard QC procedures, it is recommended that Positive and Negative Controls be run every 25 tests (twice per kit), and when changing operators within the test kit.

QA Testing Procedure

- Dispense 3 drops Reagent 1 and 3 drops Reagent 2 into Test Tube.
- Vigorously mix the control contents. Add 1 free falling drop of Control from dropper bottle.
- Place a clean swab into the Tube.
- Continue as you would for a patient sample, as instructed in the Procedure section.

Limitations of the Procedure

- The Signify Strap A Test has been categorized as CLIA waived only for the application of a qualitative detection of Group A Streptococcal antigen from throat swabs.
- The results obtained with this kit yield data that must be used only as an adjunct to other information available to the physician. The signify Strep A Test is a qualitative test for detection of Group A Streptococcal antigen. This test does not differentiate between viable and nonviable Group A Streptococci.
- The Signify Strep A Test should be used only with throat swabs. The use of swab specimens taken from other sites or the use of other samples such as saliva, sputum or urine has not been established. The quality of the test depends on the quality of the sample; proper throat swab specimens must be obtained.
- This test does not differentiate between carriers and acute infection. Pharyngitis may be caused by organisms other than Group A Streptococcus.

A negative result may be obtained if the specimen is inadequate or antigen concentration is below the sensitivity of the test.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard container and follow applicable health department disposal policies.
2. Treat all used testing apparatus as potentially infectious waste and follow your health department disposal policies.

Management guidelines

Follow specific Department for Health services Clinical Standards/Guidelines for Medical Management

Test Procedure

1. Verify the quality control, as described above, has been performed and documented.
2. Just before testing, add 3 drop Reagent 1 (pink) and 3 drops Reagent 2 to the Test Tube (the solution should turn light yellow).
3. Immediately put the swab into the Tube.
4. Vigorously mix the solution by rotating the swab forcefully against the side of the Tube at least ten (10) times. Best results are obtained when the specimen is vigorously extracted in the solution.
5. Let stand for 1 minute.
6. Express as much liquid as possible from the swab by pressing the swab firmly against the side of the Tube.
7. Discard the swab.
8. Remove LabStrip(s) from the container; re-cap container immediately.
9. Place the Absorbent End of the LabStrip into the extracted sample.
10. Read results in 5 minutes. Positive results may be read as soon as the red Control Line appears.

Interpretation of Test Results

A blue or red line that appears uneven in color density is considered a valid result. In cases of moderate or high positive specimens, some blue color behind the Test Line may be seen; as long as the Test Line and Control Line are visible, the results are valid.

Positive: A blue Test Line and a red Control Line is a positive result for the detection of Group Streptococcus antigen. Note that the blue line can be any shade of color and can be lighter or darker than the picture on the product insert instructions.

Negative: A red Control Line but no blue Test Line is a presumptive negative result.

Invalid: If after 10 minutes, no red Control Line appears or background color makes reading the Control Line impossible, the result is invalid. If this

occurs, repeat the test on a new LabStrip and contact Abbott Laboratories Customer Support Center at 1-800-323-9100.

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

References

Signify Strep A Test package insert, *no date noted.*

Group A Strep Test
by BioStar Acceava (Rapid Test)

Principle

The BioStar Acceava Strep A Test uses color immunochromatographic dipstick technology with rabbit antibodies coated on the nitrocellulose membrane. In the test procedure, a throat swab is subjected to a chemical extraction of a carbohydrate antigen unique to Group A Streptococcus. The Test Stick is then placed in the extraction mixture and the mixture migrates along the membrane. If Group A Streptococcus is present in the sample, it will form a complex with the anti-Group A Streptococcus antibody conjugated color particles. The complex will then be bound by the anti-Group A Streptococcus capture antibody and a visible blue Test Line will appear to indicate a positive result.

Specimen Collection/Treatment

1. Specimen: Acceptable – Pharyngeal swab collected with a sterile swab. Unacceptable – Specimens collected from other sources than the throat or nasopharynx**.**

2. Swabs: Acceptable – Sterile rayon or dacron tipped swab on a plastic shaft. Unacceptable – Swabs with wooden shafts, calcium alginate, or cotton tips.

3. Specimen Storage: Process swabs as soon as possible after collection. Swabs may be stored at room temperature (15-30 C) or refrigerate (2-8 C) for up to 24 hours.

4. Handling Precautions: Follow your laboratory safety guidelines in the collection, handling storage, and disposal of controls and patient specimens.

Reagents and Equipment

Reagents and materials provided:

Test Sticks – Lots of 50 to be stored at room temperature (15-30 C).
Test tubes – Lots of 50 to be stored at room temperature (15-30 C).

Sterile Swabs – Lots of 50 to be stored at room temperature (15-30 C).
Reagent 1 – One bottle of 2M Sodium Nitrite to be stored at room temperature (15-30 C).
Reagent 2 – One bottle of 0.3M Acetic Acid to be stored at room temperature (15-30 C).
Positive Control – One bottle of nonviable Group A Streptococci, 0.1% Sodium Azide to be stored at room temperature (15-30 C).
Negative Control – One bottle of nonviable Group C Streptococci, 0.1% Sodium Azide to be stored at room temperature (15-30 C).
One Package Insert

Materials required but not provided:

Timer or Watch

Storage and Stability

Store Test Sticks and reagents tightly capped at 15-30 C (59-89 F). Do not use Test Sticks or reagents after expiration date.

Safety Precautions

- BioStar Acceava Strep A is intended for in-vitro diagnostic use.
- Follow your laboratory safety guidelines in the collection, handling, storage, and disposal of controls, patient specimens and all items exposed to patient specimens.
- **Reagent 2 contains acid.** If the solution comes in contact with the skin or eyes, flush with large volumes of water.
- The Positive and Negative Controls contain Sodium Azide which may react with lead or copper plumbing to form potentially explosive metal Azide. Large quatities of water must be used to flush discarded control material down a sink.

Quality Control

Internal Procedural Controls
The BioStar Acceava Strep A Test provides three levels of prcedural controls with each test run. For daily control, BioStar recommends documenting these controls on each day of testing.

- The color of the liquid changes from pink to light yellow as you add Extraction Reagent 2 to Extraction Reagent 1. This is an internal extraction reagent control. The color change means that you mixed the extraction reagents properly. The color change also means that the reagents are functioning properly.
- The red Control Line is an internal control. The Test Stick must absorb the proper amount of sample and the Test Stick must be working properly for the red Control Line to appear. For the Test Stick to be working properly, the capillary flow must occur.
- A clear background is an internal background negative control. If no interfering substances are in the specimen and the Test Stick is working properly, the background in the Control Line area will clear. A discernible result will be seen.

External Quality Control Testing

- Each kit contains Positive and Negative Control material. The Controls are for external quality control testing. Use the Controls to test that the extraction reagent and the test Sticks are working. Also use the Controls to test that you are able to correctly perform the test procedure. If you choose, you may use Group A and non-Group A Streptococcus ATCC reference strains as controls. Some commercial controls may contain interfering additives. Therefore BioStar recommends that you do not use other commercial controls with the Acceava Strep A Test.
- In addition to your laboratory's standard QC procedures, BioStar recommends that Positive and Negative Controls be run every 25 tests, (twice per kit), and when changing operators within the test kit.

Remedial Actions

If the red Control Line does not appear, the test may be invalid. If the background does not clear and interferes with the test result, the test may be invalid. Call BioStar's Technical Support at 800-637-3717, if you experience either of these problems.

Test Procedure

- ***Extraction Procedure***
  1. Just before testing, add three (3) drops of Reagent 1 (pink to light red) and three (3) drops of Reagent 2 to the Test Tube. (The solution should turn light yellow).

2. Immediately put the swab into the Test Tube.

3. Vigorously mix the solution by rotating the swab forcefully against the side of the Tube at least 10 times. Best results are obtained when the specimen is vigorously extracted in the solution.

4. Let stand one (1) minute.

5. Express as much liquid as possible from the swab by pressing the swab firmly against the side of the Test Tube.

6. Discard the swab

- ***Assay Procedure***
  1. Remove Test Stick(s) from the container and recap the container immediately.

  2. Place the Absorbent End of the Test Stick into the extracted sample.

  3. Read the results at five (5) minutes. Positive results may be read as soon as the red Control Line appears.

Procedure Notes

Note: A blue or red line, which appears uneven in color density, is considered a valid result. In cases of moderate or high positive specimens, some blue color behind the Test Line may be seen, however as long as the Test Line and Control Line are visible, the results are valid.

Reporting and Interpreting Results

1. Positive Result: A blue Test and red Control Line is a positive result for the detection of Group A. Streptococcus antigen. Note that the blue line can be any shade of color.

2. Negative Result: A red Control Line but no blue Test Line is a presumptive negative result.

3. Invalid Result: If after 10 minutes, no red Control Line appears or background color makes reading the red Control Line impossible, the result is invalid. If this occurs, repeat the test on a new test Stick or contact BioStar's Technical Support at 800-637-3717

Test results are recorded on patient's medical chart using CH-12 form.

Limitations

- The BioStar Acceava Strep A Test has been categorized as CLIA waived only for the application of qualitative detection of Group A Streptococcal Antigen from throat swabs. The application for the confirmation of presumptive Group A Streptococcal colonies recovered from culture is not waived.
- The results obtained with this kit yield data that must be used only as an adjunct to other information available to the physician. The Acceava Strep A Test is a qualitative test for the detection of Group A Streptococcal antigen. This test does not differentiate between viable and nonviable Group A Streptococci.
- The BioStar Acceava Strep A Test should be used only with throat swabs, or colonies taken directly from a plate. The use of swab specimens taken from the other sites or the use of other samples such as saliva, sputum or urine has not been established. The quality of the test depends on the quality of the sample; proper throat swab specimens must be obtained.
- This test does not differentiate between carriers and acute infection. Pharyngitis may be caused by organisms other than Group A Streptococcus.
- A negative result may be obtained if the specimen is inadequate or antigen concentration is below the sensitivity of the test. A negative BioStar Acceava Strep A test shall be followed up with testing using the culture method.

References

BioStar Acceava Strep A (Rapid Test) package insert, date not given

Balows, Albert, et. al., Manual of Clinical Microbiology, American Society for Microbiology, Washington D.C., 5th ed., 1991.

Complete Urinalysis

A "Complete Urinalysis" consists of three parts:

1. Physical examination consisting of color, clarity, and volume
2. Chemical analysis by urine dipstick
3. Microscopic examination of the urinary sediment, as applicable.

Physical Examination

Color:

Normal urine color ranges from colorless to yellow to dark amber varying with the concentration of the urine, the diet, and medication ingested. Some disease processes can influence the color of the urine such as blood gives a pink-red color and bilirubin gives a dark yellow-green/brown color.

Clarity:

Freshly voided urine is usually clear. Specimens left at room temperature for more than 30 minutes may become cloudy due to bacterial growth and /or the precipitiation of amorphous crystals.

Volume:

State the total volume of the specimen received into the laboratory.

Urine Microscopies

Microscopic Examination of Urine Sediment

Principle

The microscopic examination of urine is a useful tool in the detection of renal and urinary tract diseases. Centrifuged urine sediment contains the insoluble elements that have accumulated in the urine as it was processed in the kidney and passed through the urinary tract. Cells from both the lining of the urinary tract (epithelial cells) and the circulating blood (RBC's and WBC's) may appear in the urine. Casts formed in the renal tubules and collecting ducts, crystals, and organisms (bacteria, yeast, and parasites) may also appear in the urine sediment.

Equipment and Supplies

Microscope
Clean microscope slide
Clean microscope coverslip
15 mL conical bottom centrifuge tube
Tube rack
Marking pen
Quality Control Material

Specimen Collection and Preparation

1. The specimen of choice for a microscopic evaluation is the first morning urine because it is the most concentrated specimen, however for screening purposes a random voided specimen (specific gravity >1.010 and pH <7.0) may be satisfactory.

2. The urine specimen must be freshly voided and examined without excessive delay to avoid cellular deterioration. If testing will be delayed the specimen may be refrigerated for a maximum of two hours. Allow the specimen to reach room temperature prior to testing.

3. Routine voided specimens are usually satisfactory for screening purposes. In the female patient there is less contamination with vaginal elements if the urine is collected as a midstream specimen. (See appendix for specimen collection techniques).

Specimen Collection Notes

1. Casts, RBC's and WBC's are expecially susceptible to lysis in specimens with specific gravity less than 1.010 and /or a pH greater than 7.0.

2. Routine voided urine may contain cellular elements from the urethral meatus and/or vaginal secretions, in situations where a less contaminated specimen is desired the clean catch technique may be more appropriate. (See appendix for specimen collection technique).

Control Storage and Use

1. Perform and document two levels of quality control every day of testing.

2. Prepare the control according to the manufacture's instructions. See the product insert for instruction.

3. Store quality control material according to manufacture's instructions found in the product insert.

4. Do not use past the expiration date.

5. Follow the testing procedure as written below.

6. Two levels of quality control must be within acceptable limits prior to reporting any patient test results.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all body fluids, uses testing apparatus as potentially infectious waste and follow your health department disposal policies.

Test Procedure

1. Verify that the daily quality control, as described above, has been performed and documented.

2. Mix the urine specimen well and pour 12 mL into a pre-labeled conical bottom tube and cap securely.

3. Perform physical/chemical (dipstick) evaluations as necessary.

4. Centrifuge at 2000 rpm for 5 minutes.

5. The supernatant fluid (clear liquid above the sediment) is poured off, and the sediment is gently re-suspended in the few drops of urine remaining.

6. Place a drop of sediment directly on a clean microscope slide by tilting the tube and allowing the drop to run down the side of the tube or transfer a drop to the slide with a pipette.

7. Cover the drop with a clean coverslip.

Examine at once, the prepared slide will dry out upon setting and the elements may appear distorted.

a. Elements are best identified in low light and with a lowered condensor. Continuous use of the fine focus adjustment is necessary while scanning.

b. Systematically scan the entire coverslip, including the edges where casts tend to congregate.

c. Examine the slide under low power (10x) magnification to locate casts. Count the number of casts in at least 10 low power fields (1pf) and average. Use high power to identify the type of cast seen.

d. Using the high power (40x) objective identify and count red blood cells (RBC"s), white blood cells (WBC's), and epithelial cells (Epi's), if present in the sediment. Count at least 10 high power fields (hpf), average. Report as # cells/hpf.

e. Also note bacteria, yeast, parasites, and /or crystals if present in the sediment. Bacteria, yeast, and parasites should be quantitated on low power. Report 1+ when on the average1/4 the microscope field is covered with bacteria, 2+ when ½ the field is covered, 3+ when ¾ the field is covered, and 4+ when the entire field is covered. Crystals are quantitated under low power. Any abnormal crystals should be correlated with the patient's history and confirmed by a reference laboratory. Large amounts of mucous should be noted.

The microscopic findings should correlate to the urine dipstick findings.

Calculations

For each element observed, average the number seen per field.

Example: 12 fields were examined and hyaline casts were seen. Find the average number of hyaline casts seen per low power field (lpf):

2+2+3+4+0+0+4+3+2+1+1+1=23 divide by 12 = 2 hyaline casts / lpf

Expected Results

Element	Reporting
Red Blood Cells	# RBC's/hpf
White Blood Cells	# WBC's/hpf
Epithelial Cells	# Epi's/ hpf
Casts / Crystals	Note amount of organisms ¼ field covered .. 1+ ½ field covered .. 2+ ¾ field covered .. 3+ All field covered .. 4+

Reporting Results

Test results are recorded on the patient's medical chart using the CH-12 form.

Procedure Notes

Commercial systems of tubes, pipettes, and slides are available to further standardize the system such as Kova by ICL Scientific and others.

Limitations of the Procedure

1. A standardized testing procedure should be followed to minimize imprecision which may occur due to the many variables in the specimen preparation technique. A standardized procedure leads to improved accuracy and reproducibility.

2. Interpretation of urine sediment requires training and practice.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

Microscopic View of Urine Sediment

Please refer to the Color Atlas of Urine Sediment following this section.

Cellular Elements (Viewed under 40 x, High Power)

1. Erythrocytes: Red Blood Cells (RBC's): pale green biconcave discs, slightly varied size (average diameter 75 m), without nucleus. Normal to find 0-2/hpf.

RBC's can become crenated in hypertonic urine and appear as small, rough cells with ruffled edges. Both smooth, folded, and crenated cells may be seen in the same specimen. Specimens from females may be contaminated with menstrual discharge. Yeast cells and oil droplets can look very similar to RBC's.

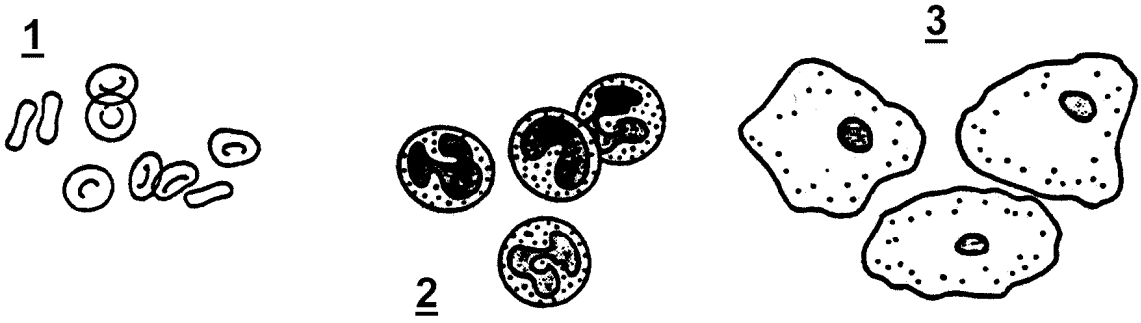
2. Leukocytes: White Blood Cells (WBC's): granular spheres, sometimes referred to as "glitter cells" because the granules appear to be moving. WBC's average 12 um diameter. Normal to find in adults 0-1/hpf and children 1-5/hpf. WBC's can look very similar to renal epithelial cells.

3. Epithelial Cell's (Epi's): Three types can be seen:

Squamous: Large, flat cells, varying in size, cells may appear folded, in sheets, or rolled up like cigars, cells originate from the urethra and vagina, prevalent in urine of females.

Bladder or Transitional: 2-4 times larger than WBC's, pear, cuboidal or columnar shape with distinct small oval to round nucleus.

Renal or Tubular: Round and slightly larger than WBC's with a single large nucleus.



Casts

Casts are cylindrical bodies made primarily of protein. They vary in diameter, have parallel sides, and flat or rounded ends. They are often hard to see, and very low lighting is required. Normal urine may contain 0-2 hyaline or granular cast/low power field. Casts are classified as follows:

1. Hyaline: colorless, homogeneous, semi-transparent, may contain a few cells, often found near the edge of the cover slip.

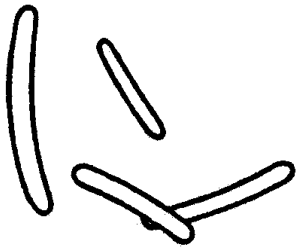
2. Fine granular: containing small grains.

3. Coarsely granular: containing small grains.

4. Waxy: opaque yellow-tan, sides and ends appear irregular or broken.

5. Cellular: containing intact WBC's, RBC's, Epi's.

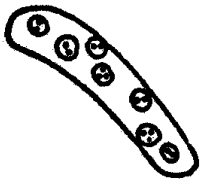
6. Fatty: containing fat droplets.



Hyaline

Cellular: RBC

Waxy



Cellular: WBC

Crystals

Normal Alkaline Urine (pH 7 or greater)

1. Amorphous phosphate crystals: small colorless granules.

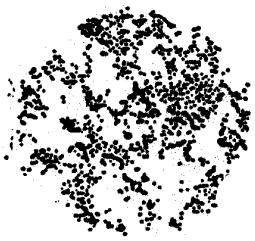
2. Ammonium magnesium phosphate (triple phosphates): crystals are colorless, three to six sided prisms ("coffin lids") also can be feathery-star-leaf shaped.

3. Calcium phosphate: slender colorless prisms with one wedge-like end.

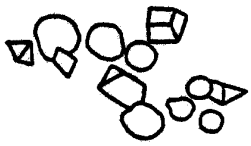
4. Calcium carbonate crystals: tiny colorless spheres or dumbbells.

5. Ammonium biurate crystals: opaque, yellow-brown, balls with thorns attached (like thorn apples), size varies, seen singly and in clusters or chains.

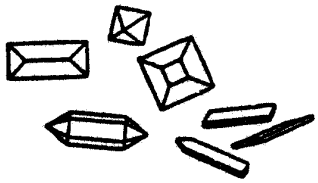
1



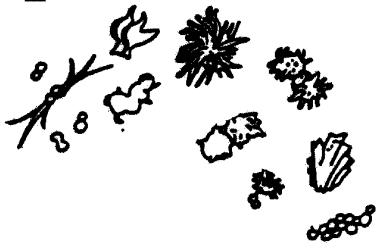
4



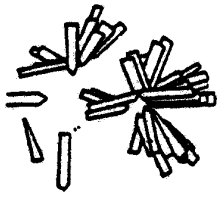
2



5



3

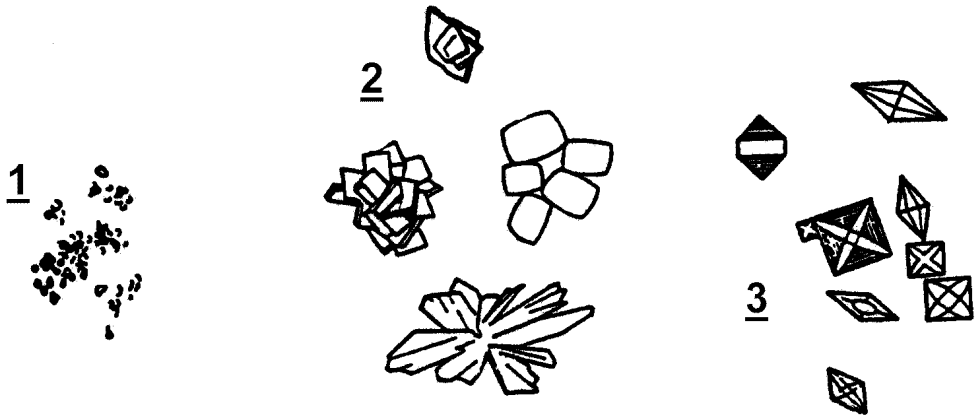


Normal Acid Urine (pH 5-6)

1. Sodium urates (amorphous urates): colorless or yellow-brown granules

2. Uric Acid: irregular shaped, colorless or yellow-brown, usually prismatic (like thick window glass)

3. Calcium oxalate: size varies, very refractile octahedral (envelopes" or can be dumbbell shaped.



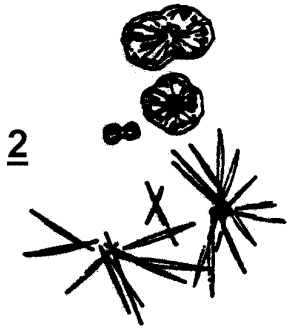
Abnormal Acid Urine

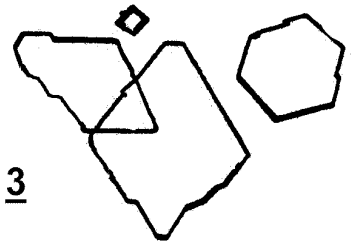
1. Cystine: highly refractile flat, colorless, hexagonal plates having one perfect fact and two imperfect neighboring facets, occur singly or in groups and clusters

2. Leucine and tyrosine are usually found together. Leucine: highly refractile, appears as oily spheres with radial striations (like a wheel). Tyrosine: highly refractile, needles arranged in sheaves (like wheat), usually appear black but can be yellow-brown.

3. Cholesterol: very thin, colorless transparent plates, large, flat, with random notched, broken edges.

4. Hippuric acid: colorless or yellow needles or prism.



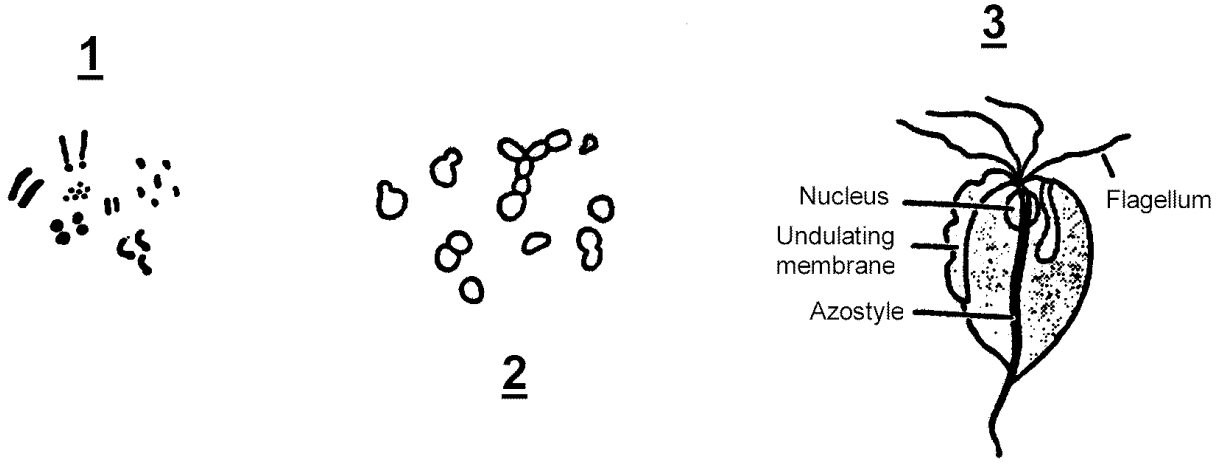


Microorganisms

1. Bacteria: originating from vaginal or fecal contamination as well as true urinary tract infection.

2. Yeast: vary in size, colorless and sometimes budding.

3. Parasites: most prevalent in both male and female is Trichomonas vaginalis, pear-shaped protozoan about the size of a WBC, may be motile (swirling, jerking, turning) by the whip-like flagella at one end.

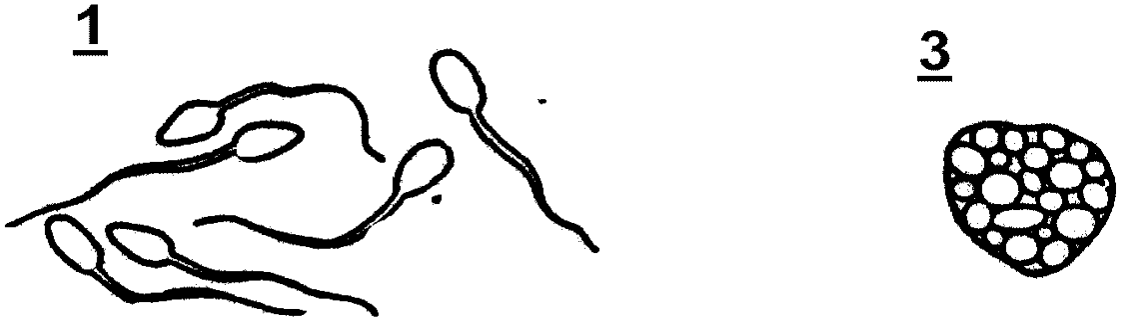


Miscellaneous

1. Spermatozoa: oval body with long delicate tail, some may still be motile, sometimes clinically significant.

2. Mucous strands: vary in size and width, have tapered ends, can be confused with casts.

3. Oval fat bodies: round refractive bodies with many small round droplets inside.



References

Boehringer-Mannheim Diagnositics, Urinalysis Today, 1987.

Henry, John B., Clinical Diagnosis & Management by Laboratory Methods, 18th ed., Saunders Co., 1991.

International Society for Clinical Laboratory Technology's, Physician Office Laboratory Technician Handbook, ISCLT St. Louis, MO, 1989.

Free, Helen M. editor, Modern Urine Chemistry, Mile Inc., Elkhart, IN, revised 1991.

National Committee for Clinical Laboratory Standards, Physician's Office Laboratory Guidelines, Vol. 12, No. 5, June 1992.

Amines or Whiff Test, vaginal fluids

Principle

Bacterial vaginosis often results from a disruption or alteration of the normal vaginal flora. It may be the cause of up to half of the cases of vaginitis seen in women. The most widely accepted clinical criteria for the diagnosis of bacterial vaginosis are a milky-homogenous vaginal discharge, a positive amine or whiff test, a raised vaginal pH, and the presence of clue cells on the wetmount examination.

The typical amine "fishy" odor may be recognized on speculum examination, the intensity of the smell increases markedly with the addition of 10% Potassium Hydroxide (KOH) solution.

It is thought that a raised vaginal pH results in the liberation of certain volatile amines such as putrescine and cadaverine—the breakdown of amino acids generated by the metabolism of the abundant bacteria found in bacterial vaginosis. These amines are responsible for the typical "fishy" odor of bacterial vaginosis.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all inoculated culture plates as potentially infectious waste and follow your health department disposal policies.

Specimen Collection and Test Procedure

1. Following the pelvic examination, drops of 10% KOH are added the vaginal secretions. Vaginal fluid may be applied to a microscope slide where drops of 10% KOH are then added.

Expected Results

2. If anaerobic bacteria are present, a "fishy" odor will be noted due to the liberation of amines.

Reporting Results

Test results are recorded on the patient's chart using the CH-12 form.

Limitations of Procedure

1. Positive tests are also found following sexual intercourse and in infections with *Trichomonas vaginalis*.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

References

Priestley, Cecilia JF and GR Kinghorn, "Bacterial Vaginosis", British Journal of Clinical Practice 50, no . 6 (September 1996): 331-34

Majeroni, MD, Barbara A., Bacterial Vaginosis: An Update, American Family Physician Journal, March 15, 1998.

American College of Obstetricians and Gynecologist (ACOG) Technical Bulletin no. 226, Vol 54, No .6, Vaginitis, Nov. 1, 1996.
http://www.aafp.org/apf/98031ap/majeroni.html

KOH Prep

In conjunction with the saline-tube wet mount procedure

Principle

KOH added to the wet mount preparations enhances visibility by acting as a clearing agent. Yeast and fungal elements become more clearly visible when the background has been cleared.

Equipment and Supplies

Gloves
Sterile cotton-tipped applicator stick
Transfer pipette
Sterile or clean test tube (option 2 only)
Clean microscope slides
Clean microscope coverslips
Sterile 10% Potassium hydroxide (10% KOH)
Microscope

Reagent Storage Instructions

KOH may crystallize over time. When significant crystallization occurs the KOH should be replaced with a fresh supply.

Precaution

KOH is a corrosive compound -- Avoid contact with the skin or eyes. In the event of contact, wash the effected area for 15 minutes with running water. Irritation may develop.

Quality Control

No commercial quality control products are available at this time.

Disposal Of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all inoculated culture plates as potentially infectious waste and follow your health department disposal policies.

Specimen Collection and Preparation

This procedure should follow the saline-tube wetmount. Refer to that procedure for further information.

Option 1

1. After completing the saline-tube wetmount procedure, using a transfer pipette place a drop of saline/specimen suspension onto a second slide and add 1 drop of 10% KOH .

2. Place a cover slip on the slide.

3. Wait 2-5 minutes for the clearing to take place and examine.

Option 2

1. After completing the saline-tube wetmount procedure, transfer 1-2 drops of the saline/specimen solution to a second test tube and add enough KOH to dissolve cellular elements (approx. 1-3 drops).

2. Gently tilt the tube to mix. Using a transfer pipette, transfer one drop to a slide, coverslip.

3. Wait 2-5 minutes for the clearing to take place and examine.

Test Procedure

Examine at once, preparations with KOH will dry out rapidly. Elements are best identified in moderate to low light and with a lowered condenser. Elements can be observed under low power (10 X) but high power (40 X) magnification is necessary for identification.

Expected Results

The elements are seen refractile or slightly colored against the cleared background.

Yeasts are non-motile, round or oval in shape, and vary in size (2-6 μm). Some yeast have pseudohyphae which are filaments with rounded ends and vary in length (20-100μm).

Reporting Results

1. Test results are recorded on the patient's chart using the CH-12 form.

2. It is acceptable when the test is read and reported by the physician for him/her to document the results on the CH-13/ CH-14 ("Health History and Physical Examination Form") under the category "Impression/Plan" as long as the following elements are present:

    (a) Patient label containing the patient's name, unique identifier, and LHD site code
    (b) Name of test/ test results
    (c) Identity of the physician
    (d) The nurse should enter the specimen collection time on the CH-12 and in the results box write "See CH-13 or See CH-14".

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

References

Finegold, Sydney M., Bailey and Scott's Diagnostic Microbiology, C.V. Mosby Co, 7th ed., 1986.

Garcia, Lynne S., Diagnostic Medical Parasitology, El Sevier Science Pub. Co., New York, 1988.

Gardner, Pierce, MD, Manual of Acute Bacterial Infections, Little, Brown and Co., 1975.

International Society for Clinical Laboratory Technology's, Physician Office Laboratory Technician Handbook, ISCLT, St. Louis Mo., 1989.

Pattillo, Doris J., Laboratory Diagnosis of Vaginitis, Center for Disease Control, Nov. 1990.

Thomason, J.L. & Gelbart, S.M., Current Concepts for Bacterial Vaginosis, UpJohn Company, Kalamazoo, MI., 1990.

Wentworth, Berttina B. Ph.D., Laboratory Methods For The Diagnosis Of Sexually Transmitted Diseases, American Public Health Association, Washington D.C., 1984.

Borchardt, K.A., et. al., A Clinical Evaluation of Trichomoniasis in San Jose, Costa Rica Using the In Pouch TV test, Genitourinary Medicine, 1992; Vol. 68; pgs. 328-330.

Wolner-Hanssen, et. al., Clinical Manifestations of Vaginal Trichomoniasis, Journal of the American Medical Association, 261, (4); pg. 571-576, 1989, Jan 27.

pH, vaginal fluids

Principle

Bacterial vaginosis often results from a disruption or alteration of the normal vaginal flora. It may be the cause of up to half of the cases of vaginitis seen in women. The most widely accepted clinical criteria for the diagnosis of bacterial vaginosis are a milky-homogenous vaginal discharge, a positive amine or whiff test, a raised vaginal pH, and the presence of clue cells on the wetmount examination.

There are three basic types of pH paper. pH meters are used for a more exact pH measurement.

1. Litmus – pretreated paper coated with an organic dye used as an indicator. There are two types of litmus paper: red and blue. Red litmus paper is used to check for basicity (red paper turns blue when touched to bases or alkaline solutions), and blue litmus paper is used to check for acidity (blue litmus paper turns red when exposed to acids).

2. Alkacid or Wide range – a drop of solution is touched to the paper and the color is approximated by comparing it to a color chart supplied with the paper. This type of paper usually spans the whole pH range (1-14) with the resulting pH value being good to about ± 1 pH unit.

Nitrazine paper is a wide range paper changing from yellow (acid) to blue (base) reading from 4.5 to 7.5 pH units using the color comparison chart provided.

3. Short range - works similar to wide range paper except it covers a much narrower range (a couple of pH units) and gives values good to about ± 0.1 units. Usually short range paper is used in conjuction with wide range paper.

Quality Control

The pH paper should be stored in a dry place, away from light, which deteriorates the paper. Whenever you suspect possible deterioration, quality control should be performed. An acid and base solution can be used to

validate the pH paper is working properly such as vinegar/ acetic acid (acid) and Potassium Hydroxide/KOH (base).

Sometimes just the outer piece may have suffered deterioration from being left on the counter overnight, tearing off and tossing 2-4 inches may correct the problem.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all inoculated culture plates as potentially infectious waste and follow your health department disposal policies.

Specimen Collection and Test Procedure

3. Following the pelvic examination, touch the pH paper to the discharge collected on the speculum.

4. Read the pH paper as indicated by the manufacturer's package insert.

Expected Results

The normal pH of vaginal secretions is less than 4.5. A pH greater than 4.5 indicates an imbalance in the vaginal ecosystem.

Reporting Results

Test results are recorded on the patient's chart using the CH-12 form.

Limitations of Procedure

2. Take care during specimen collection to avoid the cervical mucous, as cervical mucous normally has a higher pH.

3. The pH of vaginal secretions may also be increased as a result of douching, menstruation, and sexual intercourse.

4. Amniotic fluid typically turns nitrazine paper blue (alkaline). Contamination with vaginal-cervical mucus, blood, or urine may lead to a false interpretation.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

References

American College of Obstetricians and Gynecologist (ACOG) Technical Bulletin no. 226, Vol 54, No .6, Vaginitis, Nov. 1, 1996. http://www.aafp.org/apf/98031ap/majeroni.html

Majeroni, MD, Barbara A., Bacterial Vaginosis: An Update, American Family Physician Journal, March 15, 1998.

Priestley, Cecilia JF and GR Kinghorn, "Bacterial Vaginosis", British Journal of Clinical Practice 50, no . 6 (September 1996): 331-34

Estimation of pH; http://alpha.nsula.edu/departments/chemphysics/1051/pH/ph.htm and http://www.genchem.chem.wisc.edu/

Toth, MD, Peter P. and Jothivijayarani, MD, A, University of Iowa Family Practice Handbood, 3rd Edition, Chapter 8, Obstetrics: Premature Rupture of Membranes (PROM) http://www.vh.org/Providers/ClinRef/FPHandbook/Chapter08/16-8.html

Wet Mount

Saline Tube Method

Principle

Wet mount preparations are a quick and useful test to make presumptive identification of certain microbiological elements, such as budding yeast and hyphal elements, and motile trophozoites of Trichomonas associated with bacterial vaginosis.

Equipment and Supplies

Gloves
Sterile cotton-tipped applicator stick
Sterile normal physiological saline (0.85% Sodium chloride)
Transfer pipette
Sterile or clean test tube
Clean microscope slides
Clean microscope coverslips
Microscope

Quality Control

No commercial quality control products are available at this time.

Disposal of Hazards

1. Place all sharps in the appropriate biohazard sharps container and follow applicable health department disposal policies.

2. Treat all inoculated culture plates as potentially infectious waste and follow your health department disposal policies.

Specimen Collection and Preparation

(All steps including the microscopic examination should be performed at once with no delays)

5. Add a standardized volume of room temperature saline to a test tube. (0.5mL of 0.875% sodium chloride is recommended.)

6. During the pelvic examination swab suspicious areas (vaginal, cervix, or urethral), using a cotton tipped applicator stick.

7. Place the specimen soaked swab into the tube containing saline. Swish the swab to mix the specimen into the saline. Press the swab against the tube wall to expel as much specimen as possible and discard the swab. Gently tilt the tube to further mix the solution.

8. Using a transfer pipette, transfer a drop (about 36µL) of the saline/specimen solution to a glass microscope slide.

   NOTE: A second slide can be prepared adding a small drop of KOH to the slide. See the KOH procedure for more information.

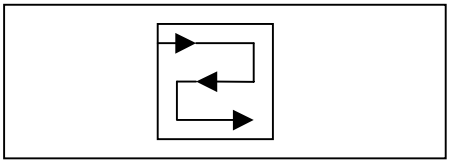
9. Place a cover slip (22 mm sq.) on the slide. Examine immediately.

Test Procedure

10. Examine at once, wet mount preparations will dry out rapidly and motile trichomonads will become labile (non-motile) under the heat of the microscope light.

    Elements are best identified in moderate to low light and with a lowered condenser. Elements can be observed under low power (10 X) but high power (40 X) magnification is necessary for identification. Ten high power fields should be examined, reporting the average range of elements viewed (such as 10- 15 WBCs/ hpf). Note the presence of trichomonads, clue cells, yeast, WBCs, bacteria, normal epithelial cells, and any parasites seen.

    A systematic method of examination is to begin in a corner and read in rows across the coverslip. Be sure to include fields in both the center and the edges of the coverslip.



Optional step

Staining the wetmount slide with urine sediment stain may be helpful in identifying some elements. The nuclear material will pick up the stain

(purple color) and create greater color contrast with the cytoplasm. If too much stain is used the whole coverslip will look dark purple. A drop of stain can be diluted in a test tube with saline to give a lighter purple color. To stain the slide, add a small drop of stain or diluted stain to the edge of the coverslipped slide. Capillary action will pull the stain under the coverslip. The wooden end of the applicator stick can be used to transfer a smaller drop of the stain to the coverslip edge. Stain will not work on the same slide where KOH has been added. The stain may slow or stop the motility of the trichomonas.

Expected Results

1. Trophozoites of Trichomonas vaginalis are highly motile (swirls, jerks, and turns) pear-shaped organisms about the size of a white blood cell, with an undulating membrane on one side (appears to vibrate), and four whip-like flagella at one end.

   Motility must be observed to distinguish trichomonads from white blood cells which may also be present. One observed motile trichomonad is enough to report their presence.

2. Yeasts are non-motile, round or oval in shape, and vary in size (2-6 5M). Some yeast has pseudohyphae, which are filaments with rounded ends and vary in length (20-1005m).

3. Clue cells are squamous vaginal epithelial cells that are covered with bacteria, giving them a granular or fringed appearance. The borders are obscured or fuzzy because of the adherence of small rods or cocci.

Reporting Results

1. Test results are recorded on the patient's chart using the CH-12 form.

2. It is acceptable when the test is read and reported by the physician for him/her to document the results on the CH-13/ CH-14 ("Health History and Physical Examination Form") under the category "Impression/Plan" as long as the following elements are present:
   a. Patient label containing the patient's name, unique identifier, and LHD site code

b. Name of test/ test results
c. Identity of the physician
d. The nurse should enter the specimen collection time on the CH-12 and in the results box write "See CH-13 or See CH-14".

Limitations of Procedure

The microbiological elements of interest must be distinguished from the normal background elements found in wet mounts such as:

1. Yeast cells may be confused with red blood cells.

2. Hyphae elements may be confused with various fabric fibers or epithelial cell wall fragments.

3. Non-motile Trichomonas sp. may resemble a white blood cell.

4. Various large rod shaped organisms seen on wet mount lying on or around squamous epithelial cells should not be confused with true clue cells.

Management Guidelines

Follow specific Department for Health Services Clinical Standards/Guidelines for Medical Management.

References

Finegold, Sydney M., Bailey and Scott's Diagnostic Microbiology, C.V. Mosby Co, 7th ed., 1986.

Garcia, Lynne S., Diagnostic Medical Parasitology, El Sevier Science Pub. Co., New York, 1988.

Gardner, Pierce, MD, Manual of Acute Bacterial Infections, Little, Brown and Co., 1975.

International Society for Clinical Laboratory Technology's, Physician Office Laboratory Technician Handbook, ISCLT, St. Louis Mo., 1989.

Pattillo, Doris J., Laboratory Diagnosis of Vaginitis, Center for Disease Control, Nov. 1990.

Thomason, J.L. & Gelbart, S.M., Current Concepts for Bacterial Vaginosis, UpJohn Company, Kalamazoo, MI., 1990.

Wentworth, Berttina B. Ph.D., Laboratory Methods For The Diagnosis Of Sexually Transmitted Diseases, American Public Health Association, Washington D.C., 1984.

Borchardt, K.A., et. al., A Clinical Evaluation of Trichomoniasis in San Jose, Costa Rica Using the In Pouch TV test, Genitourinary Medicine, 1992; Vol. 68; pgs. 328-330.

Pascoe, R Seils, et. al., Comparison Between Vaginal Swabs and Endocervical Swabs during Pelvic Examination, Journal of Adolescent Health. Vol. 15, No.3: pgs. 245-48, 1994 May.

Wolner-Hanssen, et. al., Clinical Manifestations of Vaginal Trichomoniasis, Journal of the American Medical Association, 261, (4); pg. 571-576, 1989, Jan 27.

Appendix A
Laboratory Safety

The laboratory worker should maintain a safety awareness at all times to protect her/himself as well as others.

Biological Hazards

1. Use protective coverings when handling blood, other body fluids which may contact blood, mucus membranes, or non-intact skin.

   Examples:

   Cover street clothes with a lab coat.

   Protect eyes, nose, and mouth with a full face shield or safety goggles and mask.

   Protect hands with latex gloves.

2. Wash hands thoroughly after removing latex gloves, handling laboratory specimens, and before leaving the laboratory area.

3. Remove protective clothing and gloves before leaving the lab area to prevent transfer of possible contamination into other work areas.

4. Do not eat, drink, smoke, or apply cosmetics or contact lenses within the laboratory area.

5. Avoid hand contact to mouth, nose, and eyes while working in the laboratory area. Keep pencils and instruments away from mouth.

6. Keep immunizations and skin test up to date: Hepatitis B vaccine, tuberculin skin test (chest x-ray as indicated), etc.

7. Treat all specimens as potentially infectious.

   a. Take care not to spill specimens or create aerosols.

   b. Use extreme care when removing rubber stoppers to avoid spatters and aerosols.

   c. Flame bacteriological loops carefully. Cool loop before use. Never place a hot loop on an agar surface containing bacteria.

8. Infectious waste should be handled separately from non-infectious waste.

a. Dispose of all sharp objects properly into a puncture resistant container Do not recap, break, or otherwise manipulate contaminated needles.

b. Puncture resistant containers, that are full, should be tightly sealed and disposed of properly.

c. Disinfect all bacteriology culture plates before discarding. (Example: Autoclave at 15lb pressure at 121ºC for 60 minutes or flood with 1:10 dilution of household bleach and let set 2-3 hours before discarding.)

d. Disinfect contaminated disposables before discarding. (Example: Soak in 1:10 dilution household bleach before discarding.)

e. The best solution to medical waste would be to contract with a medical waste management company to pickup and safely dispose of it for you.

9. Practice centrifuge safety.

a. Never open the lid while the centrifuge is running. Allow the centrifuge to come to a complete stop before opening to avoid contact with broken sample tubes.

b. Clean up all spills immediately with an appropriate disinfectant.

10. Clean up biological spills as soon as possible. Follow steps listed blow.

a. Use puncture resistant gloves to protect you from any sharp objects involved in the spill.

b. Absorb the spill with paper towels or other appropriate absorbent material. Using two pieces of stiff card board as shovels, carefully scoop the soaked towels and debris into an appropriate biohazard waste container.

c. Clean the site with soap and water until all visible material has been removed.

d. Disinfect the area with a freshly prepared 1:10 bleach solution of other appropriate disinfectant. This may be done by covering the area with absorbent paper towels and flooding the towels until they are "glistening wet". Allow the disinfectant to set briefly, then blot up the disinfectant soaked towels and place into an appropriate waste container.

Note: Bleach solution should be prepared fresh each day of use, as it loses its germicidal effect upon sitting.

e. Rinse the area well with water.

Chemical Hazards

1 Know the chemical you will be working with. Consult the Material Safety Data Sheet (MSDS) from the manufacturer before you begin to use the chemical to determine the physical and health hazards associated with the chemical.

Example:

Does it contain hazardous ingredients?
What are its physical/chemical characteristics?
Are there fire and explosion hazards?
Are there health hazards?
First aid procedures?
What should be done if the chemical is spilled?
How should the chemical be stored?

2 Know how to protect yourself when using chemicals.

a. Contain the chemical within the work area.

Example:

Cover the work area with absorbent paper towels.
Choose a work area with adequate ventilation.

b. Maintain a barrier between the potentially hazardous chemical and yourself by using protective clothing.

Example:

Lab coat, safety goggles, gloves, etc.

c. Be prepared to safely clean up accidental spills.

(Consult the MSDS to be prepared to react in case of an accidental spill.)

1. Assess the size of the spill.
   What is the exposure potential during clean up?
   Large spills may have an added risk of serious inhalation exposure.

Example of a large spill:

A gallon of bleach was dropped and the side split, spilling the entire contents on the floor.

The spill could create enough toxic vapors to possibly damage the lungs of the individual exposed long enough to clean up the spill.

- Evacuate the area.
- Open the doors and windows to ventilate the area.
- Limit exposure time of each individual by having several people take turns, contain and absorb the spill then allow time for the vapors to dissipate before completing the cleanup.

Example of a small spill:

A small puddle of bleach landed on the counter while measuring out bleach for a 1:10 solution.

The spill is very small and easy to manage. Possible skin irritation would be the hazard most likely encountered.

2. Contain the spill within the area where the accident occurred.
3. Wear protective clothing as applicable.
4. Use absorbent towels to contain, carefully wipe up and properly dispose of the debris.
5. Rinse the area well to remove all traces of the chemical. (In some instances, such as strong acids or bases, this would involve a neutralizing agent.)

Mechanical Hazards

1 Use proper lifting techniques putting the weight on the leg muscles to avoid back strain.

2 Avoid frayed electrical cords and overloaded outlets to prevent an electrical fire.

3 Any electrical equipment that produces a “tingle” when touched should be disconnected and sent for repair.

4 Keep electrical cords coiled and away from sink areas to prevent possibility of instrument displacement and electrical shock.

5 Use caution signs to prevent falls when floors are wet.

6 Keep walkways free of small articles such as pencils and paper clips that could cause unsure footing.

7 Avoid exposed sharp corners such as open drawers and cabinet doors.

8 Avoid carrying sharp objects without protective coverings.

9 FOLLOW ALL LOCAL FIRE AND SAFETY CODES.

References

Balows, Alvert, Chief Editor, Manual of Clinical Microbiology, 5th Edition, American Society of Microbiologists, Washington, D.C., 1991.

Baron, Ellen Jo and Finegold, Sydney M., Bailey and Scott's Diagnostic Microbiology, 8th Edition, Mosby Co., St. Louis, MO., 1986.

Kent, P.T. and Kubica, G.P., Public Health Mycobacteriology: A Guide for the Level III Lab, Centers for Disease Control, PHS, DHHS, Atlanta, GA, 1985.

Kentucky Department for Health Services, Division of Laboratory Services, Recommended Laboratory Safety Measures for County Health Departments/Centers, 1987.

Kentucky Labor Cabinet, Division of Education and Training, Hazard Communication Program, February 1988.

National Committee for Clinical Laboratory Standards, Volume 9, Number 1, Protection of Laboratory Workers from Infectious Disease Transmission by Blood, Body Fluids, and Tissue, January, 1989.

Appendix B:
Specimen Collection

Specimen Collection by Skin Puncture:

Studies have determined the maximum depth of skin puncture necessary to obtain an adequate specimen of blood without causing injury to the patient is 2.0 mm.

The usual sites selected for skin puncture are *: (See also the drawings on the next page)*

- Infant's heel (lateral or medial portions of the plantar surface)
- Greater toe of small children (lateral or medial portions of the plantar surface)
- Fingertip (lateral or medial portions of the palmer surface of the third or fourth finger)

The fingertip is the recommended site for adults and children over one year of age. Skin punctures should not be performed on the fingers of newborns or infants.
(NCCLS Vol. 15, No. 5, page D15 and Vol. 17, No. 16, page 2, section 3.1.5)

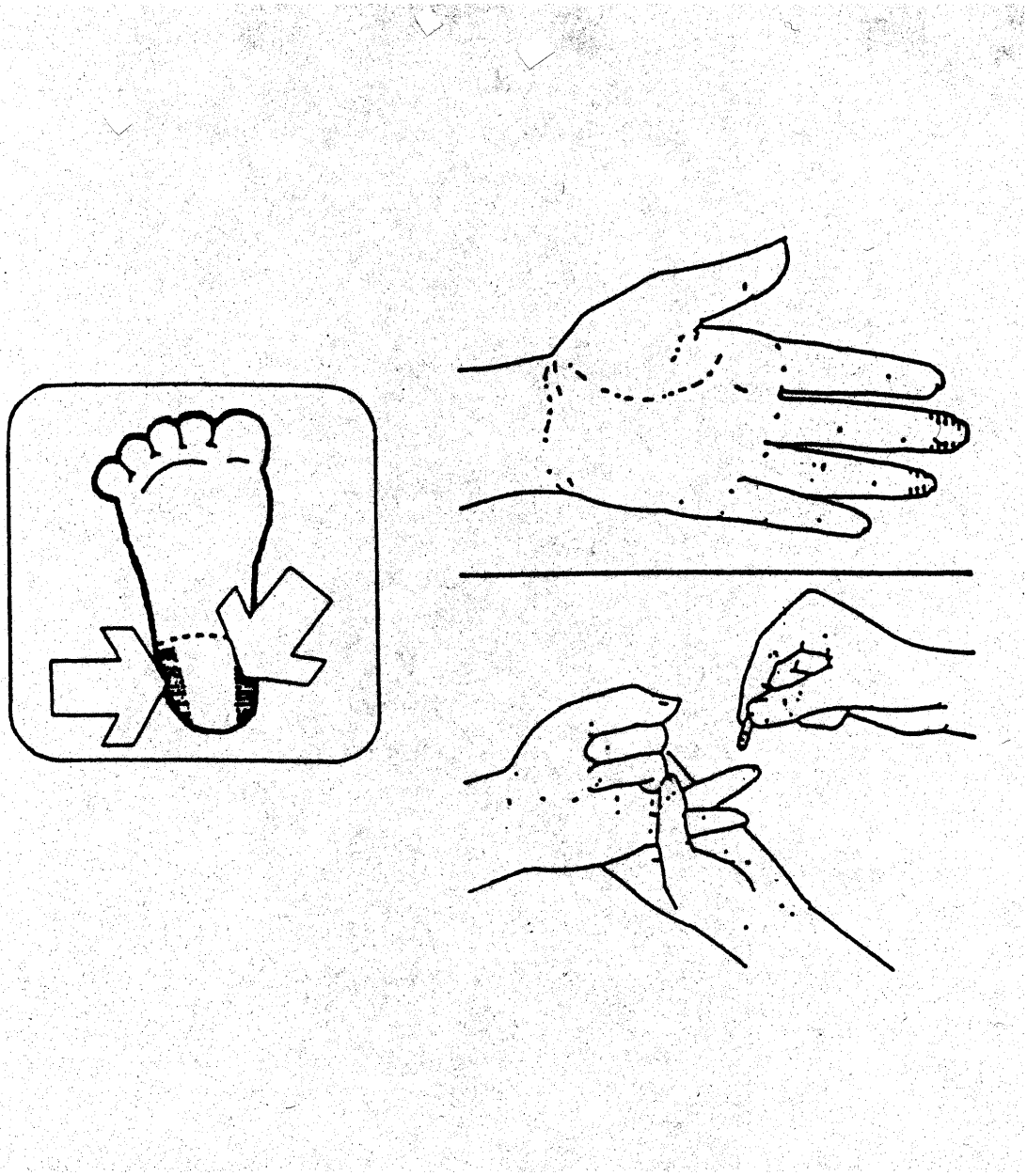
Recommended specifications for lancing devices used in the clinical setting:

1. Maximum puncture depth ≤ 2.0 mm
2. A single use, totally disposable lancing device
3. Able to deliver desired volume of specimen
4. Designed to prevent accidental re-use
5. Designed to minimize trauma and pain to the patient

Performing the skin puncture specimen collection

Lancing devices should be used as recommended by the manufacturer, consult the manufacturer's package insert for specific instructions.

- Follow all applicable safety precautions.
- Warming the puncture site will increase the blood flow. This may be accomplished by placing the hand under running water or applying a warm moistened towel for at least three minutes.
- Cleanse the puncture site with alcohol. Allow to air dry or dry with sterile cotton prior to puncturing. Residual alcohol can contaminate the specimen and promote hemolysis of the specimen.
- When using a spring loaded lancing device: pull the skin taut, apply light pressure, and activate the lancing device.
- The first drop of blood is most likely to contain excess tissue fluid and should be wiped away. Allow the next drop to form a rounded drop over the puncture site, and then lightly touch the drop to the collection device.
- Blood flow is enhanced by holding the puncture site downward and gently applying pressure to the surrounding tissue. Avoid strong repetitive pressure (milking) or scraping the collection device against the finger, these actions increase tissue fluid contamination and promote hemolysis of the specimen.
- If the blood begins to clot or run, a cotton or gauze can be used to wipe the puncture area clean. This action may promote a better blood flow from the puncture site.
- When specimen collection has been completed, apply gauze or cotton to the site to stop the bleeding.
- The specimen should be labeled with the patient's name, unique identifier, and the date and time of collection while at the collection site.



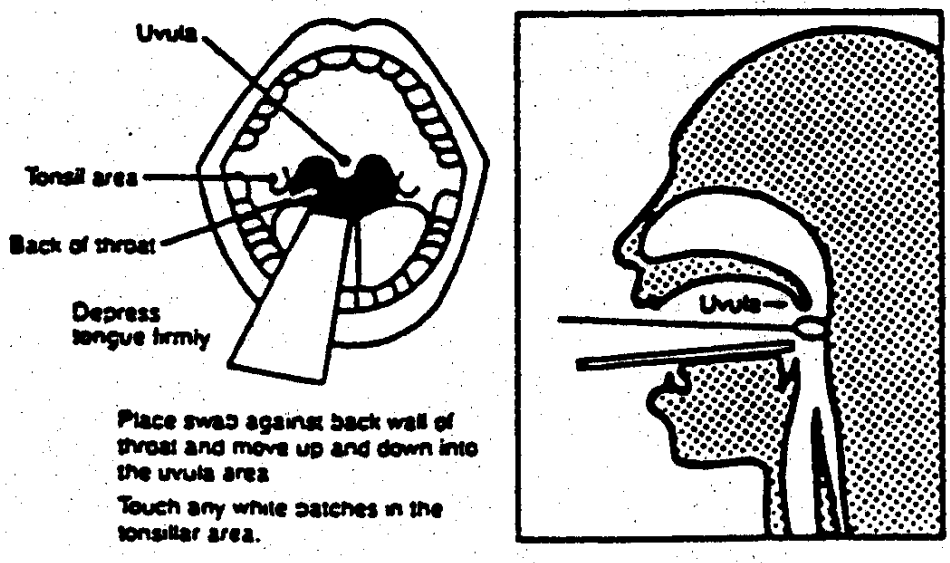
Drawing from National Committee of Clinical Standards, Physician's Office Guidelines, Vol. 12, No. 5, page D16-17.

Throat Swab Specimen Collection
for Group A Streptococcus Testing

1. Sterile swabs can be made from cotton or synthetic fibers. The test kit instructions will specify which material is most compatible with the test or provide the collection swabs within the kit.

2. Depress the tongue with a tongue blade, using a sterile dry swab sample the back of the throat and any white patches seen near the tonsillar area. The throat walls must be swabbed/ rubbed, a gentle touch will not remove the organisms adhering to the throat walls. Avoid contact with the teeth, cheeks, gums, or tongue when inserting or removing the specimen swab.

3. Begin test preparation. (See test procedure)



Drawing from National Committee of Clinical Standards, Physician's Office Guidelines, Vol. 12, No. 5, page D33.

Appendix C
Bright Field Microscopy

The basic type of laboratory microscope is the bright field microscope. The term, bright field, refers to the field of view observed when a microscopist looks into the microscope. The field of view will be filled with bright light. This appendix will be divided into three parts: the microscope parts and functions, a procedure for focusing the microscope, and routine preventative maintenance of the microscope.

Parts of the Microscope

Stand This is a mechanical term that refers to a rack, prop, or table for holding various articles. All other parts of the microscope are attached to the stand. It provides a stable platform for all the mechanical functions required for focusing the microscope. A variable intensity light source is built into the base of the stand on a modern microscope.

Stage This is a horizontal mechanical platform where the specimen is examined on a glass slide. On most microscopes the focusing is performed by raising and lowering the stage with a system of gears within the stand and which are controlled by knobs near the base of the stand. Attached to the stage is a slide holder that is moved across the stage, to center the slide, by a rack and gear mechanism and is controlled by knobs below the stage.

Condenser This is an optical lens that focuses light from the light source in the base onto the glass slide on the stage. The condenser sets in a yoke that is attached to a rack and gear mechanism located just below the stage and is controlled by a knob on the yoke.

Some condensers have an iris diaphragm to reduce the lens opening. By raising and lowering the condenser more light is brought to or withdrawn from the specimen. Closing the iris diaphragm to produce a smaller light aperture reduces excessive stray light in the viewing field making it easier to observe the specimen.

Filter A filter is a colored glass that alters the light intensity or quality for better evaluation of the specimen. It is usually desirable to have a blue filter for gram stain examinations since this filter will reduce the interfering red to yellow wavelengths of the light spectrum. A filter is usually placed on top of the condenser, but may be inserted anywhere between the light source and the stage according to the microscope design.

Nosepiece This is a disc shaped mechanical device attached to the stand above the stage. It contains several lenses (objectives) attached to the face of the nosepiece, and it is designed so the microscope can revolve the nosepiece to select the level of magnification required for specimen observation.

Objectives These are compound lenses that provide the first level of magnification. When rotated over the specimen, the objective continues the light path, now with the magnified specimen image, to be viewed by the microscopist. Objectives come in several levels of magnification, called power (X), and are selected according to the requirements for specimen observation.

Low power objectives, usually 10X, will be selected for observation of small organisms (such as insects) or to begin the focusing sequence for further observations. Mid-range objectives, usually 40-45X and called high dry objectives, are used for observations of large microorganisms (such as parasites) or for finding areas on a specimen slide that would require higher magnification. High power objectives, usually 100X, are used for observation of most bacteria. Immersion oil will be used with a high power objective (also call an oil immersion objective) to increase the objective's resolving power, the ability to render visible the fine detail of an object.

Head This device directs the light path to the eye. It may be a simple tube for monocular (one-eye) viewing of a specimen, or it may be a complex optical and mechanical device that divides the light path for binocular viewing. The head supports one or two lenses called oculars.

Ocular This is a lens that completes the magnification of the specimen image usually by 10X. Some are designed to show a larger field of view, called widefield oculars.

Focusing the Microscope

Focusing the microscope is the action of adjusting the distance from the objective to the stage by raising and lowering the stage. These adjustments are made with the knobs near the base of the stand. The larger knob makes course adjustments moving the stage a large distance per turn of the knob. The smaller knob makes finer adjustments moving the stage a small distance per turn of the knob.

In the process of focusing adjustments are made to the illumination of the specimen to bring an acceptable amount of light to the field of view. These adjustments are made by increasing or decreasing the light intensity of the light source in the base of the stand, by raising or lowering the condenser, and by opening or closing the condenser iris diaphragm.

1. Turn on the light source and adjust it to a low level of intensity. Completely open the iris diaphragm on the condenser and raise the condenser close to its highest point. Rotate the nosepiece to align the 10X objective over the light path.

2. Place the specimen slide on the stage making sure it is securely held by the slide rack. Center the specimen to the light path.

3. While looking at the distance between the stage and the objective, adjust the distance with the coarse adjustment knob until the slide is close to, but not touching, the objective.

4. Looking through the oculars, use the coarse adjustment to first lower, then raise, the stage slowly and carefully until the specimen can be observed on the slide. The adjustment distance should be slight. Care must be given when raising the stage in order to not break the specimen slide and/or damage the objective.

5. Use the fine adjustment knob to bring the specimen into sharp focus. Adjust the light intensity to an acceptable level of illumination. You may need to adjust the iris diaphragm.

   As you close the iris diaphragm stray light is reduced around the specimen image giving the appearance of more contrast to the specimen image. It also reduces the light intensity, so you must make a balance between light intensity and the desired contrast created by closing the diaphragm.

6. To switch to a mid-range objective, carefully rotate the nosepiece to bring that objective (40-45X) to the light path. This may require a small increase in the light intensity. Using the fine adjustment refocus the specimen until it is again clearly observable. You may need to readjust the iris diaphragm.

7. Switching to the high power objective (100X) requires a drop of immersion oil to focus on the specimen. To add a drop of oil to the slide, rotate the nosepiece to a position between the mid-range and high power (oil immersion) objective. Place a drop of oil on the slide.

8. Carefully rotate the nosepiece to bring the high power objective (100X) to the light path.

   Do not rotate the nosepiece to the high dry objective from this time on since the immersion oil will damage the high dry objective. The high power objective will usually rotate into the oil drop. It must make contact with the oil drop. If contact is not made with the oil drop, use the fine adjustment to bring the oil drop to the objective.

   Using the fine adjustment refocus the specimen until it is again clearly observable. This will be a very small adjustment carefully performed to prevent breaking the specimen slide and damaging the objective. The light source may need to be increased to its highest intensity, and the iris diaphragm may need to be readjusted.

9. When finished reading specimen slides, clean oil off the high power (oil immersion) objective with lens paper. **Do not scrub the lens.** First blot any excessive oil with a touch of lens paper that has been folded into a small pad. Then wipe the lens with a clean sheet of lens paper with a circular motion around the lens.

   The technique is to allow the lens paper to blot off the oil and may require wiping the lens a few times with clean portions of the paper. **Only use lens paper for cleaning objective lenses, not facial tissue paper, paper towels, or other paper wipes.**

<u>Preventative Maintenance of the Microscope</u>

The following is a general description of preventative maintenance. Your microscope manual should have a section describing procedures for the specific care of you microscope.

1. Cover the microscope when not in use.

2. Clean the oil immersion objective after reading slides.

3. Wipe dust from the condenser and oculars with a soft, lint free cloth or a fine haired brush.

4. Fingerprints and smudges on oculars, objectives, condenser, and filters should be removed with lens paper.

5. Mechanical surfaces should be wiped free of dust with a soft, lint free cloth or a fine haired brush.

6. The microscope should be cleaned and serviced annually by a reputable professional instrument service company.

There are several things not to do to a microscope:

Don't use facial tissue, paper towels, or cotton tip swabs to clean lenses. These could scratch the lenses.

Don't use lens cleaners or xylene unless you are trained to clean lenses with these reagents. Improper use of these cleaning reagents can dissolve adhesives that hold the lenses in place.

Don't use an applicator stick, a toothpick, or other sharp objects to clean lenses. These items can also scratch the lenses.

Don't take objectives and oculars apart. This may allow dust to get into the objective and may set the lenses out of alignment.

Don't take the microscope apart. This may allow dust to get into the microscope and may get the mechanism out of alignment.

Don't mix immersion oils from different manufacturers. These oils may not be compatible, causing interference in focusing of light and the specimen image.

Don't use lamps of higher wattage than recommended for your microscope illuminator. This could cause heat damage to lenses and filters.

Don't force the microscope adjustments. This will damage the gears and other parts. This includes not forcing the iris diaphragm to open or close.

A record should be maintained for all maintenance procedures performed on your microscope. This will include repairs and adjustments made by professional
service people as well as lamp changeouts and cleanings made after daily use.

References

National Committee for Clinical Laboratory Standards, Physician's Office Laboratory Guidelines, Volume 12, Number 5, June 1992.

James R. Benford, The Theory of the Microscope, Bausch & Lomb Inc., 1960.

Appendix D
Quality Control Sheets

Revision: 13 April 1994

Use this to record any service call or maintenance performed that is not recorded on the quality control log.

Instrument Maintenance Log

Name of Instrument: ____

Instrument Serial #: ________________________ Date of Purchase: ______________

Date	Initials	Documentation (Describe problem & corrective action or service call in the space below)

Quality Control

Cholesterol by Accu-check InsantPlus Instrument ID number: _____

Date	Initials	*Reagent* Lot #	Exp Date	*Control* Lev_____ Lot #_____ Expected Value	Obtained Value	Pass/Fail	Lev_____ Lot #_____ Expected Value	Obtained Value	Pass/Fail	Comments / Maintenance May Be Noted

Revision: 13 April 1994

Quality Control

Reagent Lot #_____ Exp_____Date Opened _____

_____ _____ _____

_____ _____ _____

Cholesterol by Cholestech LDX

		Calibration						**Control**							**Comments Maintenance May Be Noted**
		Self-Test		Optic Check											
Date	Initials	OK	Ch1	Ch2	Ch3	Ch4	P/F	Level	Lot#	Expir Date	Use by Date	Expect Value	Obtain Value	P/F	

Revision: 13 April 1994

Quality Control

Check Tabs Expected Values

	Min	Mean	Max

Cholesterol by Reflotron Lot Number: Expire Date:

		Calibration Check Tabs				Test Strips		Control							
Date	Initials	Obtained Values			P/F	Lot#	Exp Date	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	**Comments** / Maintenance May be Noted

Revision: 13 April 1994

Quality Control

Microcuvettes

Lot#	Expire	Use by Date	Date Opened

Glucose by HemoCue
HemoCue ID:_____

Date	Initials	Control Cuvette (+/-)	P/F	Control Level	Lot#	Expire Date	Use by Date	Expected Value	Obtain Value	P/F	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Comments / Maintenance

Quality Control Revision 1/11/02

Fecal Occult Blood Test Kit Brand Name:

Date	Initials	Test Kit Lot #	Test Kit Exp Date	Patient Information Patient Name	Patient Information Unique ID	Patient Information Test Result (+/-)	Control/ Performance Monitor Level Positive	Control/ Performance Monitor Level Negative	Control/ Performance Monitor P/F	Comments

Revision: 6 January 1997

Quality Control

Glucose by Precision QID

Instrument ID

Date	Initials	Test Strips Lot#	Expire Date	Calibration Done(√)	Controls Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Comments

Revision: 28 Feb 1997

Quality Control

Instrument ID Number:_____

Glucose by Accucheck Advantage

Date	Initials	Test Strip Lot#	Test Strip Expire Date	Controls Lev_____ Lot#_____ Exp _____ Use by _____ Expected Value	Controls Obtained Value	Controls P/F	Lev _____ Lot# _____ Exp _____ Use by _____ Expected Value	Obtained Value	P/F	Comments Maintenance may be noted

Quality Control

Revision 1/11/02

Glucose by SureStep

Instrument ID

		Test Strips			Controls														Comments
Date	Initials	Lot#	Expire Date	Calibration Code(√)	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	

Quality Control

Revision 1/11/02

Glucose by: Instrument ID

		Test Strips			Controls														Comments
Date	Initials	Lot#	Expire Date	Calibration Code(√)	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	

Revision: 13 April 1994

Quality Control

Microcuvettes

Lot#	Expire	Use by Date	Date Opened

Hemoglobin by HemoCue
HemoCue ID:_____

Date	Initials	*Control Cuvette* (+/-)	P/F	*Control* Level	Lot#	Expire Date	Use by Date	Expected Value	Obtain Value	P/F	Level	Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	*Comments /* Maintenance

Revision: 13 April 1994

Quality Control

Spun Hematocrit

Centrifuge ID:

Date	Initials	Control Level: Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Level: Lot#	Expire Date	Use by Date	Expect Value	Obtain Value	P/F	Comments Maintenance may be noted

Quality Control

Revision 1/15/02

Serum Pregnancy Test **Kit Brand Name: Mainline Confirm**

		Test Kit		Controls												
				Internal		External Level _____ Expected Value _____					Internal		External Level _____ Expected Value _____			
Date Kit QC'd	Initials	Lot #	Expire Date	C Line Present (v)	Blgrd cleared (v)	Lot #	Expire Date	Use by Date	Obtained Value	P/F	C Line Present (v)	Blgrd cleared (v)	Lot #	Expire Date	Use by Date	Obtained Value	P/F

Quality Control

Revision 1/15/02

Serum Pregnancy Test

Kit Brand Name _____

		Test Kit			Control Level _____					Level _____					Comments
Date Kit QC'd	Initials	Lot #	Expire Date	Date Kit Opened	Lot #	Expire Date	Expect Value	Obtained Value	Pass/Fail	Lot #	Expire Date	Expect Value	Obtained Value	Pass/Fail	

Quality Control

Revision 1/15/02

Urine Pregnancy Test **Kit Brand Name: Stanbio QuPID**

		Test Kit		Controls												
				Internal		External Level _____ Expected Value _____					Internal		External Level _____ Expected Value _____			
Date Kit QC'd	Initials	Lot #	Expire Date	C Line Present (v)	Blgrd cleared (v)	Lot #	Expire Date	Use by Date	Obtained Value	P/F	C Line Present (v)	Blgrd cleared (v)	Lot #	Expire Date	Use by Date	Obtained Value	P/F

Quality Control

Revision 1/15/02

Urine Pregnancy Test

Kit Brand Name: Mainline Confirm

		Test Kit		Controls													
				Internal		External Level _____ Expected Value _____					Internal		External Level _____ Expected Value _____				
Date Kit QC'd	Initials	Lot #	Expire Date	C Line Present (v)	Blgrd cleared (v)	Lot #	Expire Date	Use by Date	Obtained Value	P/F	C Line Present (v)	Blgrd cleared (v)	Lot #	Expire Date	Use by Date	Obtained Value	P/F

Quality Control

Revision 1/15/02

Urine Pregnancy Test

Kit Brand Name

_____

		Test Kit			Control Level _____					Level _____					Comments
Date Kit QC'd	Initials	Lot #	Expire Date	Date Kit Opened	Lot #	Expire Date	Expect Value	Obtained Value	Pass/Fail	Lot #	Expire Date	Expect Value	Obtained Value	Pass/Fail	

Quality Control

Revised 1/15/02

Group A Strep Testing by Abbott SIGNIFY (waived)	**External Controls** (perform every 25 tests) Lot #_____ Expire Date _____ Expected Value _____ Lot #_____ Expire Date _____ Expected Value _____

		Test Kit		Patient Information		External Controls		Built in Controls		
Date of Test	Analyst initials	Lot #	Exp. Date	Patient Name	Unique ID	Obtained Value (Pos or Neg)	Pass/Fail (P or F)	Ex. Rgt. Lt Yellow (v)	Red Control Line seen (v)	Bkgd. Cleared (v)

Quality Control

Revision 1/15/02

Strept Group A

Kit Brand Name _____ Control Name _____

		Test Kit		External Controls *(At lot # write "swab" when kit swab controls used)* **Level** _____					**Level** _____					Built-in Controls		
Date	Initials	Lot #	Expire Date	Lot #	Expire Date	Expect Value	Obtain Value	Pass/ Fail	Lot #	Expire Date	Expect Value	Obtain Value	Pass/ Fail	ExSl'n Green (v)	Blue Line at **C** (v)	Bkgrd OK (v)

Quality Control Record

Revision 1/15/02

Kit Lot Number _____

Acceva Strep A

Kit Expire Date _____

			Patient Information		Internal Quality Control			External Quality Control			Comments
Tests #	**Date of Test**	**Analyst Initials**	**Patient Name**	**Patient Unique ID**	**Reagent Color Change**	**Red Control Line**	**Clear Negative Backgrd**	*Perform external quality control when kit is opened and again ½ way thru kit.*			
					Pink to Yellow v	Present **v**	Clear v	**Results Pos QC**	**Results Neg QC**	P/F	

Quality Control

Revised 1/15/02

Urine Dipstick

Brand Name ___________________________

Date	Init	Test Lot #	Strip Exp Date	Control Lev _____ Lot #___________ Exp_________										P/F	Lev _____ Lot #___________ Exp_________										P/F	Comments
				SG	Leu	Nit	pH	Pro	Glu	Ket	Uro	Bil	Bld		SG	Leu	Nit	pH	Pro	Glu	Ket	Uro	Bil	Bld		
Control expected value																										

Quality Control

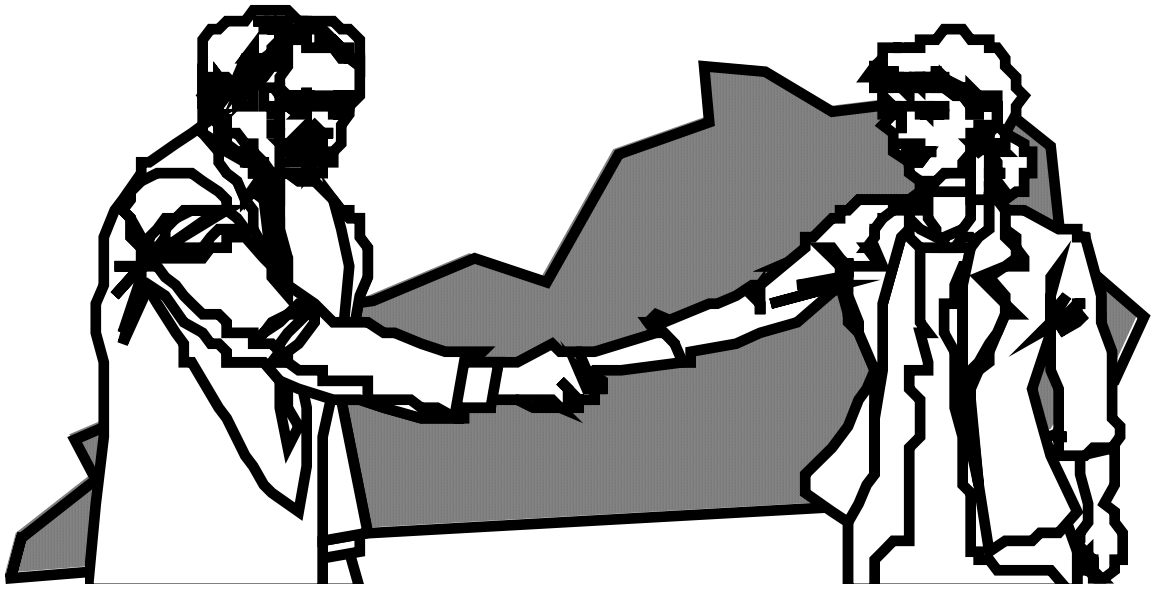
Revised 1/15/02

Urine Microscopies

Brand Name:_____

		Level _____			**Lot #** _____			**Exp.** _____			**Level** _____			**Lot #** _____			**Exp.** _____			Comments
Date	Initial	RBC	WBC	EPI	Casts	Crys-tals	Bact	yeast	Para-sites	P/F	RBC	WBC	EPI	Casts	Crys-tals	Bact	yeast	Para-sites	P/F	
Control Expect-ed value																				Each control lot enter QC expected value

LOCAL PUBLIC HEALTH LABORATORIES OF KENTUCKY



QUALITY ASSURANCE PLAN

Local Public Health Laboratories of Kentucky

QUALITY ASSURANCE PLAN

Table of Contents

LOCAL PUBLIC HEALTH LABORATORIES OF KENTUCKY
QUALITY ASSURANCE PLAN

INTRODUCTION

The laboratories which comprise the Local Public Health Laboratories of Kentucky (PHLOK) consider quality assurance (QA) to be an ongoing process that encompasses all facets of the laboratory's testing and support functions. These include specimen collection, test analysis and result reporting. QA also extends to the interaction of each member of Local PHLOK with its clients including physicians, nutritionists, environmentalists, clinics, hospitals, state and other local governmental agencies, and other laboratories.

QA OBJECTIVES

- Monitor and evaluate quality of services
- Identify and correct problems
- Assure the accurate, reliable and prompt reporting of test results
- Assure the adequacy and competency of the staff
- Continuously improve the quality of the Local PHLOK's services to its clients

RESPONSIBILITY

The laboratory director and the management team are responsible for the overall quality of all laboratory services. Responsibility for the daily quality of specific laboratory sites is delegated to the co-directors of each site.

SCOPE OF LABORATORY SERVICES

The Local PHLOK is a group of local public health laboratories joined together on a multiple site certificate in compliance with the federal Clinical Laboratory Improvement Amendments of 1988. Limited public health testing consists of a total of 15 tests from the moderate and waived categories. The 15 tests included on the Local PHLOK multiple site certificate are listed in Appendix A.

LABORATORY WIDE QUALITY ASSURANCE COMMITTEE

Each county or district which participates in the multiple site certificate has a QA Committee consisting of the following: the QA coordinator, co-director, and at least one representative from every site within the county or district. The committee meets at least quarterly. Following the QA committee meeting, each site reports on its QA activities by sending meeting minutes to the Laboratory Director for review.

The co-directors should assure that all activities required of the QA committee are performed The co-directors can also serve as QA coordinator.

QA ACTIVITIES IN COMPLIANCE WITH CLIA '88

Part I 493.1703

The laboratory will monitor and evaluate:

- The criteria for the procedures for specimen handling.

The criteria for specimen collection and rejection for each procedure can be found in the Local PHLOK's standard

operating procedure manual (SOPM), when applicable.

- The information required on the test requisition and report forms:

  Any revision of information printed on test and requisition forms (CH-12) will proceed through a protocol of review by the director, co-director, and the technical consultants.

- The information required to be maintained on the test records.

  Each co-director or designated test site supervisor will review a sampling of patient records (CH-12) monthly to ensure the following information is included:

  a) Patient's name or other unique identification and the mechanism/number that identifies the specimen to that patient.

  b) The test(s) performed.

  c) The date and time of collection.

  d) The condition and disposition of unsatisfactory specimens.

  e) The identity (initials) of the laboratory personnel who performed the test(s).

  f) Test results and units, if applicable.

- The criteria for the referral of specimens.

Local health department policy will establish the criteria for the referral of specimens to another laboratory. These criteria will be available to the personnel responsible for referring specimens. These criteria will be reviewed by the co-director when problems are identified by laboratory staff.

- The criteria for specimen rejection.

  Criteria for the rejection of specimens can be found in the Local PHLOK SOPM.

  The judgment as to whether a specimen is acceptable according to specified criteria must be made by the laboratorian performing the test. When the reliability of a specimen is questionable, the final decision should be made by the supervisor or the co-director.

  The use and appropriateness of the criteria will be reviewed as problems are identified by personnel.

- Turnaround-Time for reporting of test results.

  The director will establish turnaround-times (TAT's) for each of the 15 procedures. Turnaround-times for the 15 tests are found in Appendix B.

  If TATs are not met, an incident report shall be filed. (The Incident Report Form and incident reporting protocol can be found in Appendix C).

- Accuracy and reliability of test reporting systems, appropriate storage of records, and retrieval of test results.

Once a year the Technical Consultant will retrieve a sampling of patient test reports and check for:

a. Accuracy of laboratory-supplied information
b. Method of storage of reports
c. Time required to retrieve the reports

These reports will be from the previous two years of files. Ninety-nine percent of the laboratory-supplied information included on the report form should be correct and retrieval time should be within 4 hours. The consultant will return a copy of the Record Search Form with the on-site visit summary. When completed by the technical consultant and returned, this form should be filed or kept in the QA manual.

A blank copy of the Record Search Form can be found in Appendix D.

Part II 493.1705

The laboratory will monitor and evaluate the remedial actions taken:

. When test methods fail
. When test equipment fails
. When patient results are outside the reportable range
. When a population reference range is found to be inappropriate.
. When controls are out of range.
. When calibration is unacceptable.
. When established time-frames cannot be met.

. When reported results are found to be in error.

Remedial actions are outlined in the Local PHLOK's procedure manual. When any of the above situations are not resolved by the remedial actions, an incident report should be filed as outlined in the protocol found in

Appendix C.

Incident reports are reviewed within three months of the date filed. These reports indicate any control material, reagents, media, equipment, test methods, and calibration procedures that consistently do not give expected results or do not meet performance specifications. Completed incident reports will be compiled and reviewed by the QA Committee at each county or district health department.

When incident report reviews indicate that the remedial actions have been ineffective, the co-director and testing personnel will meet to determine new remedial actions. The technical consultant may be involved, if desired.

Part III 493.1707

The laboratory will monitor and evaluate the effectiveness of corrective actions taken when proficiency testing results are unacceptable, unsatisfactory, or unsuccessful. A representative of the multiple site certificate will participate in a HCFA-approved proficiency testing (PT) program for each category of testing listed on the certificate. In addition, all sites will participate in the method validation survey program provided by the Division of Laboratory Services. Participants will be

monitored for each category in which they provide testing, where practical.

All PT results are reviewed by the director, the co-director, and technical consultant as they are submitted to each site. In the event of 1) an unacceptable result for an analyte or 2) an unsatisfactory performance for specialty/subspecialty, an incident report form must be filed by the co-director within five working days.

Part IV 493.1709

The laboratory will monitor and evaluate twice a year all methods for the same tests done on different instruments.

Participants in the joint certificate will be monitored and evaluated twice a year to verify the consistency of all methods for the same tests done on different instruments. The technical consultants will monitor method validation survey results to assure consistency of performance among all participants on the certificate and file an incident report as appropriate. The incident report will be routed to the local site for input on incident description and review.

Part V 493.1711

The laboratory will identify and evaluate patient test results that appear to be inconsistent with relevant patient information.

The laboratorian performing the test will ensure that results/ specimens are consistent with patient demographics. If any

inconsistencies occur, the test system will be checked and the specimens will be repeated. No results will be reported until the situation is resolved.

Part VI 493.1713

The laboratory will evaluate the policies and procedures for assuring the competency of its employees.

Once each year the QA committee will review the policies and procedures used to evaluate the competency of employees performing moderate complexity testing. A performance evaluation must be performed on all employees performing moderate complexity tests at least annually, and after the initial six months for the new employee. A Performance Checklist for each procedure can be found in Appendix E.

Part VII 493.1715

The laboratory will identify and document problems that occur as a result of communication breakdowns.

All instructional information that is distributed to clients of Local PHLOK sites is reviewed by the co-director for accuracy, clarity, conciseness, and completeness.

The Incident Report Form is a tool for documenting problems which occur. It provides a means for review and eventual resolution of the problem. An Incident Report Form and the incident reporting protocol can be found in Appendix C.

When a consistent problem occurs as a result of communication breakdown, the appropriate co-director and other related staff will work with the QA committee to minimize the recurrence of these problems.

Part VIII 493.1717

The Local PHLOK will document all complaints and problems reported to the laboratory and will investigate those complaints.

Any complaint/problem (whether internal or external) received or observed by any laboratory employee will be reported using the Incident Report Form. See Appendix C.

When corrective actions are necessary to decrease problems with a procedure (either pre-analytical, analytical, or post-analytical) they will be documented on the Incident Report Form. The effectiveness of these actions will be evaluated in the three-month review required on the Incident Report Form.

Part IX 493.1719

The laboratory will document, assess, and discuss problems identified during any quality assurance reviews with all staff members.

The QA Committee quarterly meeting minutes will be distributed to all staff members or posted on the laboratory bulletin board for staff review.

The Incident Report Form requires that the reported incident be discussed with all appropriate staff members. A resolution is achieved through the combined effort of the

appropriate co-director(s), supervisor(s), technical consultant(s), other involved staff, and when necessary, the director.

Part X 493.1721

The laboratory will maintain all documentation of any QA activity.

Any specific site's QA activity documentation will be maintained at that site.

Any county- or district-wide QA activity, including Incident Reports, will be maintained at the office of the designated county or district QA coordinator.